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ABSTRACT

This document is an instructional module package prepared in objective form for use by an instructor familiar with multiple tube and membrane filter techniques for determining total coliform concentration of a water supply. Included are objectives, instructor guides, student handouts and transparency masters. This module considers proper laboratory practices, proper sampling, equipment and media preparation, test procedures and data interpretation. (Author/RH)

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TOTAL COLIFORM DETERMINATIONS

Training Module 5.205.3.77

Prepared for the

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by

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September, 1977

SE 024 249

The mention of trade names, or use of manufacturers technical bulletins, diagrams depicting specific equipment, or the commercial product in this module is for illustration purposes, and does not constitute endorsement or recommendation for use by Kirkwood Community College nor by the Iowa Department of Environmental Quality.

Module No:	Module Title: Total Coliform Determination in Drinking Water
Approx. Time: 2½ hours	Submodule Titles: 1. Multiple Tube Technique 2. Membrane Filter Technique

Overall Objectives:

Upon completion of this course, the participant should be able to determine the total coliform level in drinking water by the multiple tube and/or the membrane filter technique.

Instructional Aids:

Handout A
Handout B
Transparancies
Necessary Laboratory Reagents and Equipment

Instructional Approach:

Discussion
Demonstration
Laboratory practice

References:

1. Standard Methods for the Examination of Water and Wastewater, 14th Edition.
2. Simplified Procedures for Water Examination (AWWA-M-12).
3. Basic Laboratory Skills module

Class Assignments:
Read Handouts A & B
Test a drinking water sample by the multiple tube method.
Test a drinking water sample by the membrane filter technique.

Module No:	Topic: SUMMARY

Instructor Notes:	Instructor Outline:
1. Handout A Transparancies	1. Discuss and demonstrate the multiple tube method of total coliform determination.
2. Supply all necessary equipment for the participant to complete on actual total coliform determination by the multiple tube method. *	2. Have participant analyze a given sample for total coliform density.
3. Handout B Transparancies	3. Discuss and demonstrate the membrane filter technique of total coliform determination.
4. Supply all necessary equipment for the participant to complete an actual total coliform determination by the membrane filter technique. *	4. Have participants analyze a given sample for total coliform density.
The use of technical bulletins from the major manufacturers may be helpful in supplementing information in student handouts.	
* List of basic laboratory reagents and equipment necessary included in student handout.	

Module No:	Module Title: Total Coliform Determination in Drinking Water
	Submodule Title: Multiple Tube Method
Approx. Time: 2 hours	Topic: Introduction

Objectives:

Upon completion of this module, the participant should be able to:

1. Describe the need for testing for the presence of total coliform bacteria in drinking water.
2. Give the total coliform standards for drinking water as set forth by the U.S.E.P.A.

Instructional Aids:

Handout A - Section #1

Instructional Approach:

Discussion

References:

1. Standard Methods for the Examination of Water and Wastewater, 14th Edition.
2. Simplified Procedures for Water Examination (AWWA-M-12)
3. Basic Laboratory Skills module.

Class Assignments:

1. Read Handout A (Section #1)
2. The learner will provide the number (Figure only) of samples required to be tested for their distribution system.

Module No:	Topic: Introduction

Instructor Notes:	Instructor Outline:
Handout A (Section #1)	1. Discuss the need for the determination for Total Coliform. 2. Discuss the number of samples to be tested as required by law for Total Coliform Determination. 3. Discuss the bacteriological standards for drinking water supplies required by USEPA. 4. Ask learner to determine the number of samples to be tested for his/her city or town.

Module No:	Module Title: Total Coliform Determination in Drinking Water
	Submodule Title: Multiple Tube Method
Approx. Time: 1½ hours	Topic: Applicable Basic Laboratory Skills Review

Objectives:

Upon completion of this module, the participant should be able to:

1. Describe necessity for laboratory practices including:
   a. Setting laboratory rules
   b. Proper glassware cleaning & storage
   c. Aseptic technique
2. Identify and properly use major laboratory equipment
3. Explain proper sampling and sample dilution techniques

Instructional Aids:

Handout A -(Appendix A, B, & C)

Transparancy #1

Necessary Laboratory Reagents and Equipment

Instructional Approach:

Lecture
Discussion
Laboratory Practice

References:

1. Standard Methods for the Examination of Water and Wastewater, 14th Edition.
2. Simplified Procedures for Water Examination (AWWA-M-12).
3. Basic Laboratory Skills module.

Class Assignments:

Read Handout Appendix A, B, C
Practice using major laboratory equipment

Module No:	Topic: Applicable Basic Laboratory Skills Review

Instructor Notes:	Instructor Outline:
Review basic laboratory skills module	
Handout A (Appendix A)	1. Discuss the importance of setting laboratory rules on: a. Clothing b. Safety & safety equipment c. Record keeping
Handout A (Appendix A)	2. Discuss and demonstrate proper methods of glassware cleaning, glassware storage and aseptic technique.
Transparancy #1	3. List and discuss the use of major laboratory equipment including: a. Autoclave b. Sterilizing oven c. Incubators d. Distillation unit e. Glassware washer f. Refrigerator
Handout A (Appendix B & C)	4. Discuss sampling and sample dilution
Put emphasis on areas of procedure where errors occur and their affect on outcome.	5. Have participants practice using major equipment.

Module No:	Module Title: Total Coliform Determination in Drinking Water
	Submodule Title: Multiple Tube Method
Approx. Time: 3 hours	Topic: Equipment and Media Preparation

Objectives:

Upon completion of this module, the participant should be able to:

1. List the bench equipment needed to plant and transfer water samples.
2. Describe and demonstrate preparation and use of:

   a. Culture media and culture tubes
   b. Sterile dilution water

Instructional Aids:

Transparancy #2
Handout A (Section #2)
Demonstration
Necessary Laboratory Reagents and Equipment

Instructional Approach:

Discussion
Demonstration
Laboratory Practice

References:

1. Standard Methods for the Examination of Water and Wastewater, 14th Edition.
2. Simplified Procedures for Water Examination (AWWA-M-12)
3. Basic Laboratory Skills module

Class Assignments:
Read handout A - Section #2
Prepare growth media
Prepare culture tubes

Module No:	Topic: Equipment and Media Preparation

Instructor Notes:	Instructor Outline:
Handout A (Section #2) Demonstration Transparancy #2	1. List and demonstrate use of bench equipment needed to complete test procedure. 2. Discuss preparation, use, and storage of sterile dilution water. 3. Discuss and demonstrate preparation of: a. Culture media b. Culture tubes 4. Have learner practice preparation of culture media and tubes.

Module No:	Module Title: Total Coliform Determination in Drinking Water
	Submodule Title: Multiple Tube Method
Approx. Time: 6 hours	Topic: Test Procedure

Objectives:

Upon completion of this module, the participant should be able to:

1. List the characteristics of a positive test.
2. Discuss and demonstrate proper technique for:
   a. Planting sample
   b. Transferring growth from presumptive to confirmatory media
   c. Recording data obtained from analysis
3. Discuss importance of proper incubation times and temperatures

Instructional Aids:

Handout A (Section #3)

Transparancies #3, 4, 5

Necessary Laboratory Reagents and Equipment

Instructional Approach:

Discussion
Demonstration
Laboratory Practice

References:

1. Standard Methods for the Examination of Water and Wastewater, 14th Edition.
2. Simplified Procedures for Water Examination (AWWA-M-12)
3. Basic Laboratory Skills module

Class Assignments:

Read handout A - Section #3
Practice tube inoculations
1. Planting sample using pipet
2. Transferring growth using loop
Practice recording data

Module No:	Topic: Test Procedure

Instructor Notes:	Instructor Outline:
Handout A - Section #3 Demonstration Transparancies # 3, 4, 5	1. Discuss test procedure 2. Demonstrate: a. Use of pipet for planting sample b. Use of loop for transferring growth 3. Describe appearance of a positive test result and what to do with it. 4. Demonstrate proper method of recording test data. 5. Discuss and demonstrate proper disposal of used culture tubes. 6. Have learner plant samples in 15 tubes and transfer the positive growth tubes and record data on worksheet.

Module No:	Module Title: Total Coliform Determination in Drinking Water
	Submodule Title: Multiple Tube Method
Approx. Time: 2 hours	Topic: Data Interpretations

Objectives:

Upon completion of this module, the participant should be able to:

1. Determine the total coliform level found in the drinking water sample tested.
2. Describe what to do if the results of the analysis are not within the normal range.
3. Given a set of test results determine whether the water is bacteriologically safe to drink.

Instructional Aids:

Handout A (Section #4)

Transparancy #6

Instructional Approach:

Discussion

References:

1. Standard Methods for the Examination of Water and Wastewater, 14th Edition.
2. Simplified Procedures for Water Examination (AWWA-M-12)
3. Basic Laboratory Skills module

Class Assignments:

1. Read Handout A - Section #4
2. Practice calculation and data interpretation.

Module No:	Topic: Data Interpretations

Instructor Notes:	Instructor Outline:
Handout A (Section #4) Transparancy #6	1. Discuss the acceptable Total Coliform Level 2. Discuss what to do if the results of the analysis are not within the Normal Range including: a. Increasing the frequency of testing b. Reporting to Supervisor 3. Discuss factors which erroneously affect test results a. Errors in sampling b. Erros in Lab technique c. Errors in calculation 4. Discuss whether any given water is bacteriologically safe to drink.

Module No:	Module Title: Total Coliform Determination in Drinking Water
	Submodule Title: Membrane Filter Technique
Approx. Time: ½ hour	Topic: Introduction

Objectives:

Upon completion of this module the participants should be able to:

1. Explain the importance of testing for the presence of total coliforms in drinking water.
2. Describe:
   a. The total coliform group
   b. Water quality standards

Instructional Aids:

Handout B (Section #1)

Instructional Approach:

Discussion

References:

1. Standard Methods for the Examination of Water and Wastewater, 14th Edition.
2. Basic laboratory skills module.

Class Assignments:

Read Handout B - Section #1

Module No:	Topic: Introduction

Instructor Notes:	Instructor Outline:
Handout B - Section #1	1. Discuss the relationship between total coliforms and pathogenic (disease causing) bacteria. 2. Describe the morphology of the total coliform bacteria including: a. Size b. Shape c. Colony morphology on m-Endo agar 3. Discuss total coliform standards for drinking water including history. 4. Ask learner to determine the number of samples to be for his/her city or town.

Module No:	Module Title: Total Coliform Determination in Drinking Water
	Submodule Title: Membrane Filter Technique
Approx. Time: 1½ hours	Topic: Applicable Basic Skills Review

Objectives:

Upon completion of this module the participant should be able to:

1. Describe the necessity for laboratory practices including:
   a. Setting laboratory rules
   b. Proper glassware cleaning and storage
   c. Aseptic technique
2. Identify and properly use major laboratory equipment.
3. Explain proper sampling and sample dilution techniques.

Instructional Aids:

Handout B (Appendixes A, B, & C)

Transparanc y #1

Necessary Laboratory Reagents and Equipment

Instructional Approach:

Lecture
Discussion
Laboratory Practice

References:

1. Standard Methods for the Examination of Water and Wastewater, 14th Edition.
2. Basic laboratory skills module

Class Assignments:

1. Read Handout B, appendixes A, B, & C
2. Practice using major laboratory equipment

Module No:	Topic: Applicable Basic Skills Review

Instructor Notes:	Instructor Outline:
Review Basic Laboratory Skills module	
Handout B - Appendix A	1. Discuss the importance of setting laboratory rules on: a. Clothing b. Safety and safety equipment c. Record keeping
	2. Discuss and demonstrate proper methods of glassware cleaning, glassware storage, and aseptic technique.
	3. List and discuss the use of major laboratory equipment including: a. Autoclave b. Sterilizing oven c. Incubators d. Distillation unit e. Glassware washer f. Refrigerator
Transparancy #1	
Handout B - Appendixes B & C	4. Discuss sampling and sample dilution.
Put emphasis on areas of procedure where errors occur and their affect on outcome.	5. Have participants practice using major equipment.

Module No:	Module Title: Total Coliform Determination in Drinking Water
	Submodule Title: Membrane Filter Technique
Approx. Time: 3 hours	Topic: Equipment and Media Preparation

Objectives:

Upon completion of this module the participant should be able to:

1. List the bench equipment and expendables needed to filter the water sample, culture the membrane, and count the colonies.
2. Describe and/or demonstrate proper preparation of:
   a. Culture media
   b. Sterile dilution water
   c. Equipment and expendables for test

Instructional Aids:

Handout B - Section #2
Transparancy #7
Demonstration
Necessary Laboratory Reagents and Equipment

Instructional Approach:

Discussion
Demonstration
Laboratory Practice

References:

1. Standard Methods for the Examination of Water and Wastewater, 14th Edition.
2. Basic laboratory skills module

Class Assignments:

1. Read Handout B - Section #2
2. Practice preparing:
   a. Culture media
   b. Sterile dilution water
3. Practice preparing bench equipment and expendables.

Module No:	Topic: Equipment and Media Preparation

Instructor Notes:	Instructor Outline:
Handout B - Section #2 Transparancy #7	1. List and demonstrate use of bench equipment and expendables needed to complete test procedure. 2. Discuss preparation, use, and storage of sterile dilution water. 3. Discuss and demonstrate preparation of culture media. 4. Have participant practice: a. Preparing culture media b. Preparing dilution water c. Wrapping bench equipment for sterilizi

Module No:	Module Title: Total Coliform Determination in Drinking Water
	Submodule Title: Membrane Filter Technique
Approx. Time: 4 hours	Topic: Membrane Filtration Procedure

Objectives:

Upon completion of this module the participant should be able to discuss and/or demonstrate proper technique for:

1. Dispensing media (both broth and agar)
2. Assembling filtration equipment
3. Filtering any volume of sample size
4. Plating and incubating inoculated membrane filter

Instructional Aids:

Handout B - Section #3
Transparancies #8 & 9
Demonstration
Necessary Laboratory Reagents and Equipment

Instructional Approach:

Demonstration
Laboratory Practice

References:

1. Standard Methods for the Examination of Water and Wastewater, 14th Edition.
2. Basic laboratory skills module

Class Assignments:

Read Handout B - Section #3
Practice procedure by assembling equipment, filtering several dilutions of a water sample, and plating and incubating the cultured membrane filter.

Module No:	Topic: Membrane Filtration Procedure

Instructor Notes:	Instructor Outline:
Handout B - Section #3 Transparancy #8 Demonstration	Discuss and demonstrate preparation of work area. 1. Disinfection 2. Equipment assembly 3. Dispensing m-Endo broth and m-Endo agar
Handout B - Section #3 Demonstration and transparancy	Discuss and demonstrate sample filtration 1. Placing membrane in funnel 2. Adding sample 3. Filtering and rinsing 4. Removal of filter from funnel
Handout B - Section #3 Transparancy #9 Demonstration and transparancy	Discuss and demonstrate culturing of membrane 1. Placing membrane on broth and agar based media 2. Incubation Have students practice all of the above.

Module No:	Module Title: Total Coliform Determination in Drinking Water
	Submodule Title: Membrane Filter Technique
Approx. Time: 2 hours	Topic: Counting Procedure

Objectives:

Upon completion of this module, the participant should be able to:

1. Determine by examination, which membrane in a sample set requires counting.
2. Describe proper counting methodology.
3. Demonstrate ability to differentiate between total coliform and non-total coliform colonies and count total coliform colonies accurately.

Instructional Aids:

Handout B - Section #4
Transparancy #10 & 11
Demonstration
Necessary Laboratory Reagents and Equipment

Instructional Approach:

Discussion
Demonstration
Laboratory Practice

References:

1. Standard Methods for the Examination of Water and Wastewater, 14th Edition.
2. Simplified Procedures for Water Examination (AWWA-M-12).
3. Basic Laboratory Skills module

Class Assignments:

Read Handout B - Section #4
Practice counting total coliform colonies on membrane filters

Module No:	Topic: Counting Procedure

Instructor Notes:	Instructor Outline:
Handout B - Section #4 Transparancies #10 & 11 Demonstration	Discuss and demonstrate how to choose the corre membrane to count and proper counting methodology. Discuss colony differentiation including: a. Colony color b. Colony shape c. Colony size Have students practice counting colonies on membrane filters.

Module No:	Module Title: Total Coliform Determination in Drinking Water
	Submodule Title: Membrane Filter Technique
Approx. Time: 2 hours	Topic: Data Interpretation and Evaluation

Objectives:

Upon completion of this module, the participant should be able to:

1. Compute number of total coliforms per 100 ml. given dilution mls. filtered, and total coliform count.
2. Determine whether sample meets standards given water source, table of acceptable limits, and total coliform count.
3. Identify necessary action given a result not meeting standards.

Instructional Aids:

Handout B - Section #5
Transparancy #12
Demonstration

Instructional Approach:

Discussion B - Section #5
Demonstration
Practice Problem

References:

1. Standard Methods for the Examination of Water and Wastewater, 14th Edition.
2. Simplified Procedures for Water Examination (AWWA-M-12).
3. Basic Laboratory Skills module.

Class Assignments:

Read Handout B - Section #5
Calculate # of total coliforms per 100 ml. sample from membrane counted.
Answer sample problem question.

Module No:	Topic: Data Interpretation and Evaluation

Instructor Notes:	Instructor Outline:
Handout B - Section #5 Transparancy #12	Demonstrate calculating # total coliforms per 100 mls. Have students do practice problem. Have students calculate # total coliforms per 100 mls. for sample they filtered. Discuss whether sample meets standards and what to do if it does not.

TOTAL COLIFORM DETERMINATION IN DRINKING WATER

Transparancy List

Transparancy #1: Sample dilution

Transparancy #2: MPN equipment

Transparancy #3: Pipet and loop

Transparancy #4: Positive test

Transparancy #5: Recording MPN test data

Transparancy #6: MPN chart

Transparancy #7: MF equipment

Transparancy #8: MF equipment set up

Transparancy #9: Plating method

Transparancy #10: Choose correct MF to count

Transparancy #11: Counting methodology

Transparancy #12: Calculating count per 100 mls.

HANDOUT A

MULTIPLE TUBE TECHNIQUE FOR THE DETERMINATION OF COLIFORMS IN DRINKING WATER

SECTION I: INTRODUCTION TO COLIFORM TESTING

I. COLIFORMS ARE A GROUP OF BACTERIA

A. Need oxygen to survive

B. Rod shaped

C. Gram negative

D. Ferment the sugar lactose with gas production within 48 hrs. at 35° C.

E. Found in fecal matter and decaying plant matter.

F. See Figure #1 for size comparison.

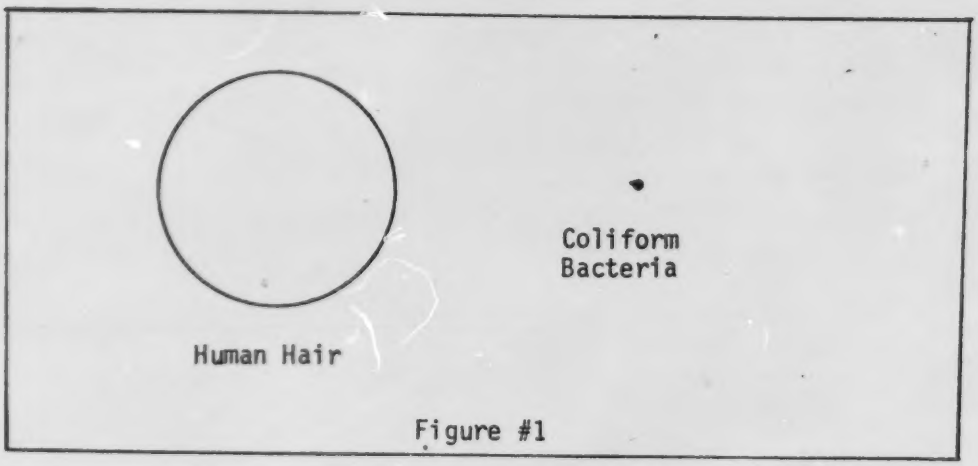


Figure #1

II. COLIFORMS IN DRINKING WATER MAY INDICATE

A. Fecal matter present, therefore disease causing organisms present.

B. Insufficient chlorination, therefore disease causing organisms still alive also.

III. WATER QUALITY STANDARDS FOR DRINKING WATER

A. Not more than 10% of all portions tested during any month shall show coliform bacteria present.

B. The coliform determination shall not show coliform bacteria present in:

1. Three or more portions in more than one sample per month when less than 20 samples per month are examined.

2. Three or more portions in more than 5% of the samples per month when more than 20 samples per month are examined.

3. Three or more portions in two consecutive samples.

C. Number of samples which must be taken monthly.

Population served:	Minimum number of samples per month
25 to 2,500	2
2,501 to 3,300	3
3,301 to 4,101	4
4,100 to 4,900	5
4,901 to 5,800	6
5,801 to 6,700	7
6,701 to 7,600	8
7,601 to 8,500	9
8,501 to 9,400	10
9,401 to 10,300	11
10,301 to 11,100	12
11,101 to 12,000	13
12,001 to 12,900	14
12,901 to 13,700	15
13,701 to 14,600	16
14,601 to 15,500	17
15,501 to 16,300	18
16,301 to 17,200	19
17,201 to 18,100	20
18,101 to 18,900	21
18,901 to 19,800	22
19,801 to 20,700	23
20,701 to 21,500	24
21,501 to 22,300	25
22,301 to 23,200	26
23,201 to 24,000	27
24,001 to 24,900	28
24,901 to 25,000	29
25,001 to 28,000	30
28,001 to 33,000	35
33,001 to 37,000	40
37,001 to 41,000	45
41,001 to 46,000	50
46,001 to 50,000	55
50,001 to 54,000	60
54,001 to 59,000	65
59,001 to 64,000	70
64,001 to 70,000	75
70,001 to 76,000	80
76,001 to 83,000	85
83,001 to 90,000	90
90,001 to 96,000	95
96,001 to 111,000	100
111,001 to 130,000	110
130,001 to 160,000	120
160,001 to 190,000	130
190,001 to 220,000	140
220,001 to 250,000	150
250,001 to 290,000	160
290,001 to 320,000	170
320,001 to 360,000	180

SECTION 2: BENCH EQUIPMENT AND MEDIA PREPARATION

I. LIST OF BENCH EQUIPMENT, MEDIA, AND REAGENTS

A. Bench Equipment

1. Hot plate
2. Balance with 0.5 gm. sensitivity
3. pH meter
4. Bunsen type burner
5. Pipet soaking jar

B. Glassware

1. 1 l. erlynmeyer flasks
2. Sample bottles
3. Graduated cylinders
4. 100 ml. dillution blanks
5. Test tubes - 150 x 18 mm - borosilicate glass plus caps
6. Test tubes - 75 x 10 mm - borosilicate glass
7. Pipets - 10 ml & 1 ml - calibrated in 0.1 ml. - T.D. or Mohr
   a. Sterile, disposable, cotton plugged, individually wrapped - or -
   b. Borosilicate glass with aluminum or steel can for sterilizing in.

C. Expendables

1. Non-absorbent cotton
2. Brown Kraft wrapping paper
3. Aluminum foil
4. Rubber gloves
5. Paper towels
6. Sponge
7. Marking pens

D. Safety Equipment

1. Fire extinguisher
2. Fire blanket
3. First aid kit
4. Emergency shower
5. Emergency eye wash

E. Reagents and Media

1. Disinfectant
2. Peptone or KH_2PO_4
3. 1 N NaOH
4. 1 N HCl
5. Lactose broth or Lauryl tryptose sulfate broth
6. Brilliant green bile broth
7. Distilled water

II. BENCH EQUIPMENT PREPARATION & FUNCTION

A. Hot plate

1. Keep top clean for even heat
2. Used to heat solutions to aid in dissolution

B. Balance with 0.5 gm. sensitivity

1. Keep clean and checked for accuracy
2. Used to weigh dry media and reagents

C. pH meter

1. Check for accuracy with known standards
2. Used for checking pH of prepared media

D. Bunsen type burner

1. Clean gas jet to prevent clogging
2. Adjust for a blue flame with good cone
3. Used for sterilization of inoculating loop

E. Pipet soaking jar

1. Clean weekly to remove old pipets and spent disinfection.

2. Holds used pipets until cleaned or disposed of.

III. GLASSWARE PREPARATION & FUNCTION

A. Function of each item listed

1. 1 l. erlynmeyer flasks used for media preparation must be washed and dried.

2. Graduated cylinders used for measuring liquid volumes must be washed and dried.

3. 100 ml. dilution blanks

a. Washed and dried

b. Filled with 99 ml. sterile distilled buffered water.

c. Sterilized

d. Used to dilute samples if necessary

4. 18 x 150 mm test tubes + caps

a. Washed and dried

b. Filled with growth media

c. Capped and sterilized

d. Used to grow bacteria

5. 10 x 75 mm test tubes

a. Invested inside 18 x 150 mm filled test tubes prior to sterilization.

b. Used to trap gas produced by bacterial growth.

6. Pipets used for measuring sample

a. Sterile disposable pipets need no preparation but must be stored in a clean dry place.

b. Borosilicate glass must be washed, dried, plugged and sterilized in proper container.

IV. REAGENT AND MEDIA PREPARATION AND FUNCTION

A. Use distilled water only

B. Sterile distilled buffered water - 2 types

1. Phosphate buffered water

a. Stock solution

1. Dissolve 34 gms. KH_2PO_4 in 500 mls. distilled water in a volumetric flask.

2. Adjust to pH 7.2 with 1 N NaOH

3. Dilute to 1 l. with distilled water

b. To make buffered water for sample dilution

1. Add 1.25 mls. stock to 1 l. distilled water

2. Mix, dispense & sterilize

2. Peptone dilution water

a. Stock solution

1. Dissolve 10 gms. peptone in 100 mls. water

2. To store sterilize 15 min. at 121° C. in an autoclave and store in refrigerator.

3. Discard if it becomes cloudy

b. To make dilution water

1. Add 10 ml. stock to 1 l. distilled water

2. Mix, dispense & sterilize

3. Sterilization of buffered and dilution water

a. Dispense 99 mls. plus 4 mls. (to allow for evaporation) in 100 ml. dilution blanks.

b. Sterilize in an autoclave for 20 min. at 121° C. (15 psi)

c. Use slow exhaust

d. Sterilize with caps loose

e. Tighten caps when removed from autoclave.

C. Sodium Thiosulfate Solution

1. Stock solution

   a. Weigh 10 gms. of Sodium thiosulfate

   b. Dissolve in 50 - 60 mls. distilled water in a 100 ml. volumetric flask.

   c. Add distilled water to bring to a final volume of 100 mls.

   d. Transfer to a stoppered, 100 ml. labeled bottle and store in refrigerator.

2. For use as a dechlorinating agent

   a. Transfer 0.1 ml. (for each 40 oz. capacity) to sample bottle with 1 ml. pipet.

D. Lauryl tryptose sulfate broth (LTSB) for presumptive test

1. Order in amounts to fit needs

   a. 1 lb. bottle will make enough media for 120 samples

   b. Available in 1/4 lb. amounts

2. Keep bottle tightly closed

   a. Dehydrated media is hygroscopic

   b. Caked media must be discarded

3. Prepare

   a. According to manufacturer's instructions

   b. In amounts applicable to use

   c. Adjust pH if necessary

4. Dispense 10 ml. ± 0.5 ml. into each clean, dry 150 x 18 mm test tube

5. Insert 1 clean, dry 75 x 10 mm test tube open end down into larger tube.

6. Cap large tube

7. Sterilize

   a. Within 1 hr. of preparation

   b. Cycle of 15 min. at 121° C. in an autoclave set for slow exhause

   c. Remove from autoclave immediately upon completion of cycle

8. Cool to room temperature and check pH
    a. pH = 6.8 - 7.0
    b. Discard if not within limits
9. Store in cool place for not more than 1 month

E. Brilliant green bile broth (BG) for confirming test
1. Order in amounts to fit needs
    a. 1 lb. bottle will make enough media for 1,100 confirmations
    b. Available in 1/4 lb. amounts
2. Keep bottle tightly closed
    a. Dehydrated media is hygroscopic
    b. Caked media must be discarded
3. Prepare
    a. According to manufacturer's instructions
    b. In amounts applicable to use
    c. Adjust pH if necessary
4. Dispense 10 ml ± 0.5 ml. into each clean, dry 150 x 18 mm test tube
5. Insert 1 clean, dry 75 x 10 mm test tube open end down into larger tube
6. Cap large tube
7. Sterilize
    a. Within 1 hr. of preparation
    b. Cycle of 15 min. at 121° C. in an autoclave
    c. Remove from autoclave immediately upon completion of cycle
8. Cool to room temperature and check pH
    a. pH = 7.2
    b. Discard if not 7.2
9. Store in cool place for not more than 1 month

SECTION 3: MULTIPLE TUBE PROCEDURE

I. DATA SHEET PREPARATION

II. WORK AREA PREPARATION

A. Wash hands and disinfect work bench top

1. Lowers possibility of sample contamination leading to duplication of work.

B. Assemble and label culture tubes

1. Place 5 tubes of lauryl tryptose sulfate broth in rack for each sample to be tested.

2. Label tubes

a. Sample number

b. Sample volume inoculated

c. Position of tube in series of five

III. SAMPLE INOCULATION, INCUBATION ETC.

A. Inoculate tubes

1. Shake sample vigorously

2. Deliver 10 mls. sample to each of the 5 tubes in the series

3. Use sterile 10 ml. pipets to deliver sample

4. Use aseptic technique

B. Swirl tubes gently to mix

C. For polluted waters

1. Inoculate submultiples of 10 ml.

2. Use media prepared according to manufacturer's instructions for 1 and 0.1 ml. sample volumes.

3. Label and inoculate series of 5 as with the 10 ml. volume samples.

D. Incubate 24 hrs. at 35 ± 0.5° C.

1. After 24 hrs. check for gas production

a. No gas incubate additional 24 hrs. at 35 ± 0.5° C.

b. Positive gas production

1. Record on data sheet as positive

2. Confirm results by transferring loopful to BG broth

a. Use aseptic technique

b. Use 3 mm loop

c. Label BG tube to correspond to positive LTSB tube

d. Incubate BG tube 48 hrs. at 35 ± 0.5° C.

E. Re-Incubated LTSB tubes

1. No gas production after additional 24 hrs.

a. No further action

b. Record as negative on data sheet

2. Positive gas production

a. Record on data sheet as positive

b. Confirm results by transferring loopful to BG broth

1. Use aseptic technique

2. Use 3 mm loop

3. Label BG tube to correspond to positive LTSB tube

4. Incubate BG tube 48 hrs. at 35 ± 0.5° C.

F. After BG tubes incubated 48 hrs.

1. No gas production

a. No further action

b. Record as negative on data sheet

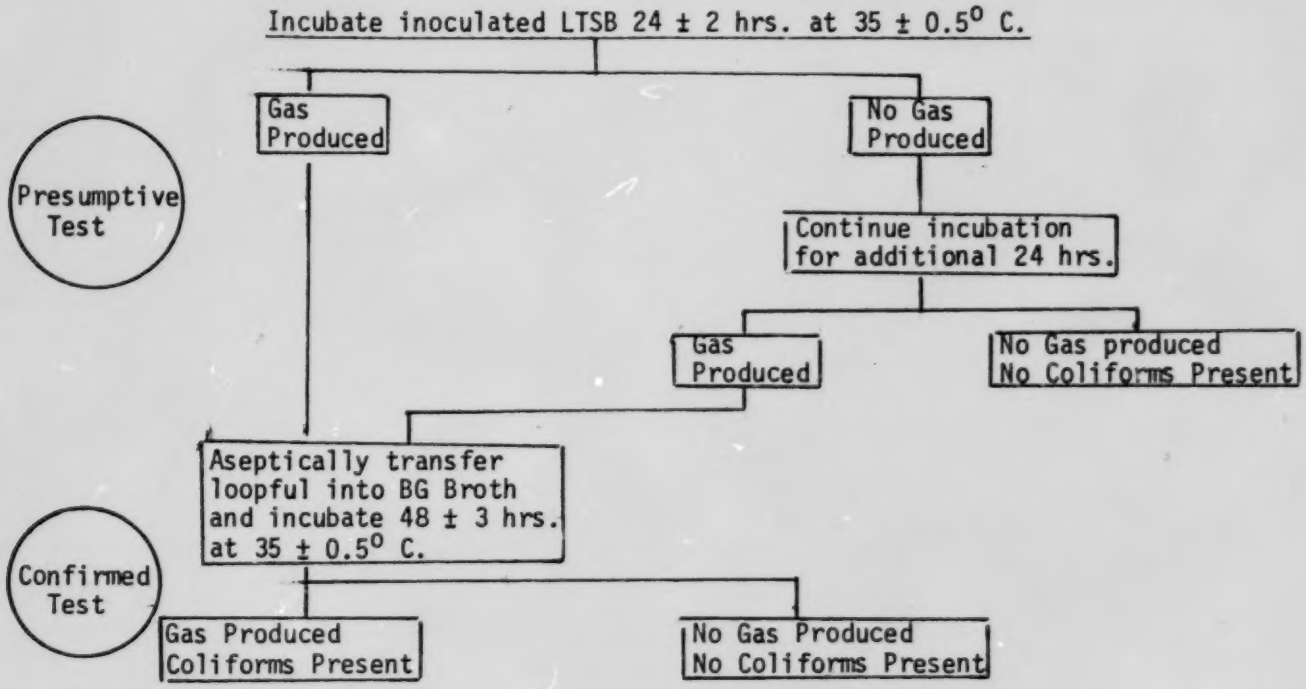
2. Gas production

a. Record on data sheet as positive

b. Indicates coliform bacteria present

c. May be further confirmed by methods described in "Standard Methods for the Examination of Water and Wastewater".

IV. SAMPLE INOCULATION AND INCUBATION - SCHEMATIC


Incubate inoculated LTSB 24 ± 2 hrs. at 35 ± 0.5° C.
Gas Produced
No Gas Produced
Presumptive Test
Continue incubation for additional 24 hrs.
Gas Produced
No Gas produced No Coliforms Present
Aseptically transfer loopful into BG Broth and incubate 48 ± 3 hrs. at 35 ± 0.5° C.
Confirmed Test
Gas Produced Coliforms Present
No Gas Produced No Coliforms Present

SECTION 4: PROCESSING USED GLASSWARE

I. CONTAMINATED BUT UNCULTURED GLASSWARE

- A. Sterilization unnecessary
- B. Empty contents down drain
- C. Wash, rinse, dry as previously described
- D. Prepare for next testing series
  1. Prepare
  2. Wrap or package
  3. Sterilize

II. GLASSWARE CONTAINING CULTURES

- A. Sterilize in an autoclave
- B. Empty contents down drain
- C. Wash, rinse and dry as previously described
- D. Prepare for next testing series
  1. Prepare
  2. Wrap, package, cap etc.
  3. Sterilize

III. DISPOSABLES

- A. Discard in polypropylene bag
- B. Sterilize in autoclave
- C. Dispose of in garbage

SECTION 5: DATA INTERPRETATION & EVALUATION

I. RECORD THE NUMBER OF GAS POSITIVE TUBES FROM THE CONFIRMED TEST FOR EACH SERIES OF 5 TUBES ON DATA SHEET.

II. NUMBER OF COLIFORM BACTERIA INDICATED BY TEST RESULTS

A. When 5 tubes of 10 ml. each are inoculated

# of Tubes Giving Confirmed Positive Reaction out of 5 of 10 ml. Each	MPN Indes. /100 ml.	95% Confidence Limits	
		Lower	Upper
0	2.2	0	6.0
1	2.2	0.1	12.6
2	5.1	0.5	19.2
3	9.2	1.6	29.4
4	16	3.3	52.9
5	16	8.0	Infinite

B. When multiple dilutions are used use chart in "Standard Methods for the Examination of Water and Wastewater".

III. THE WATER WILL BE CONSIDERED UNSAFE TO DRINK IF:

A. More than 10% of all portions tested during any month show coliform bacteria present.

B. If the coliform determination shows coliform bacteria present in:

1. Three or more portions in more than one sample per month when less than 20 samples per month are examined.

2. Three or more portions in more than 5% of the samples per month when more than 20 samples per month are examined.

3. Three or more portions in two consecutive samples.

APPENDIX A - LABORATORY PREPARATION

I. SETTING LABORATORY RULES

A. Dress Code

1. Must wear lab coat or apron at all times.
2. Shoes must have full foot protection
3. Long hair must be tied back
4. Must wear protective clothing where applicable
   a. Goggles or safety glasses
   b. Asbestos gloves

B. Safety Equipment

1. General Equipment
   a. Fire extinguisher
   b. Fire blanket
   c. First aid kit
   d. Emergency shower
   e. Emergency eye wash
2. Personal equipment for each employee
   a. Lab coat or apron
   b. Goggles
   c. Asbestos gloves
3. Safety rules
   a. Must be set and enforced by supervisor
   b. All accidents must be reported to supervisor

C. Record Keeping

1. Must be maintained at all times.
2. Should include all:

a. Purchase records

b. Equipment specifications, warranties, maintenance and instruction manuals.

c. Accident reports

d. Testing data

e. Pertinent communications

f. Employee records

II. LABORATORY CLEANLINESS

A. Types of disinfectants

1. 70% Ethanol

2. Phenols i.e. O-Syl

3. Quaterniary ammonium compounds

4. Halogen compounds

5. Activated sialdehyde i.e. cidex

B. Use of disinfectants

1. Weekly

a. Wipe down all shelves removing all glassware and books

b. Wipe down all incubators, inside and outside.

c. Wipe out inside of autoclave.

2. Daily

a. Wipe down tops of all counters, large pieces of equipment

3. Immediately before testing disinfect work area

4. Immediately disinfect spills

C. Sources of Contamination

1. Dirt around lab

2. Spilled samples or cultures

3. Un-autoclaved bacterial garbage

4. Chemical contamination from use of glassware for both Chemistry testing and Bacterial testing.

III. GLASSWARE WASHING

A. All glassware must be thoroughly washed in non-toxic detergent

1. i.e. Alconox

2. Removes bacterial scum from glassware

B. Rinse 6 - 12 times in hot tap water.

1. Removes detergent residue

2. Residue is harmful to bacteria

C. Final rinse 1 - 3 times in distilled water

1. Removes mineral residue from tap water.

2. Prevents water spotting.

D. Air Dry

1. Any spot indicates dirt

2. Rewash before using

IV. PACKAGING EQUIPMENT AND STERILIZATION

A. Reasons for packaging

1. Creates a bacteria barrier

2. Allows for storage of sterile equipment

B. Proper labeling

1. Define contents

2. Date to aid in equipment rotation

C. Sterilization of equipment - 2 Acceptable Methods

1. Autoclave

a. All rubber, metal and glassware and some plastics.

b. Normal cycle 15 min. 15 121° C.

c. Exhaust rapidly

2. Hot air sterilizing oven

a. Dry glassware and metal objects only

b. Normal cycle 1 hr. at 170° C.

c. Allow to cool before use

d. Package pipets in metal containers

e. Package other equipment with aluminum foil

V. MAJOR LABORATORY EQUIPMENT

A. Autoclave

1. Before using read and follow manufacturers installation use and maintenance instructions and safety precautions.

2. Normal sterilization = 15 psi yielding 121° C. for 15 min.

3. Use to sterilze liquids and non-heat sensitive equipment.

a. Most plastics are not autoclavable and sterilized by manufacturer.

b. Sterilized media and reagents must be removed from autoclave as soon as possible after autoclave is opened.

c. Glassware may be sterilized in autoclave but must be allowed to dry before removing from autoclave.

B. Hot Air Sterilizing Oven

1. Before using read and follow manufacturers installation, use, and maintenance instructions and safety precautions.

2. Normal Sterilization = 1 hour at 180° C.

3. Use to sterilize glass and metal only.

a. Rubber and plastics will melt.

b. Liquids will evaporate and grow media components will be destroyed.

C. 35° Incubator

1. Before using read and follow manufacturers installation and maintenance instructions and safety precautions.

2. Place in permanent location.

a. Out of drafts and direct sunlight.

b. Convenient to laboratory bench and electrical outlet.

3. Install thermometer

a. NBS (National Bureau of Standards) certified thermometer.

b. Mercury bulb of thermometer should be suspended in bottle filled with water.

c. Locate centrally in incubator.

4. Install shallow pan of water in bottom of incubator

a. Maintains condition of saturated relative humidity required in bacteriological incubator.

b. Check daily and fill as necessary to keep water in pan at all times.

5. Adjust temp. to $35^{0} \pm 0.5^{0}$ C.

a. Follow manufacturers instructions

b. Allow 1 hr. between temperature adjustments.

c. Record temp. of incubator daily.

D. Water Distillation and Deionizing Unit

1. Before using, read and follow manufacturers installation, use and maintenance instructions and safety precautions.

2. Produces reagent grade water for use in making reagents and media and rinsing glassware.

E. Refrigerator

1. Set to maintain a 4^{0} C. temperature.

2. Use to hold samples waiting to be tested and to store some prepared media and reagents.

F. Glassware washer

1. Before using, read and follow manufacturers installation, use and maintenance instructions and safety precautions.

2. Automatically washes and rinses glassware.

3. Do not use home dishwasher as it does not have proper plumbing.

APPENDIX B - COLLECTING SAMPLES FOR BACTERIOLOGICAL EXAMINATION

I. EQUIPMENT PREPARATION

A. Sample bottles must be:

1. At least 100 ml. capacity with a large neck opening.
2. Thoroughly cleaned with detergent, rinsed 6 times in hot tap water, rinsed finally in distilled-deionized water, then air dried.
3. Free from spots, scum, chips, cracks, excessive scratches and other damage on which bacteria may lodge.
4. Closed with preferably an all glass ground cap closure (but screw caps can be used providing liners are free from contamination and provide a non-leaking seal.
5. Sterilized in an autoclave at 121° C. for 15 min. with Kraft paper or tin foil hood covering caps and necks of bottles and slip of paper between bottleneck and glass stopper to prevent glass stopper from sticking.

B. Bottles intended for use in collection of chlorinated samples must have a 10% sodium thiosulfate solution added at the rate of 0.1 ml. for each 4 oz. bottle prior to sterilization and sterilized in bottle.

C. Labels must be:

1. Clean and unused.
2. Attached to bottle by a means not affected by water (i.e. string or wire.)

D. Label markers must be:

1. Permanent type not affected by water.
2. Able to mark on label.

E. Sampling devices must be in working condition and properly maintained.

F. Germicide must be available to clean up spills but must not come in contact with sample or any equipment touched by sample.

G. Rubber gloves must fit and not be punctured.

H. Ice chest for transporting sample must be:

1. Sufficient size to accommodate all samples.
2. Undamaged with tight cover so cold temperature can be maintained inside.

3. Filled with enough ice to quickly chill sample but little or no free water.

I. Refrigerator must be set at 2 - 10° C. and used if samples are not examined upon immediate return to lab.

II. SAMPLE COLLECTION

A. To take sample from spigot or tap:

1. Find spigot with direct main connection

2. Put on rubber gloves.

3. Flush spigot at full flow for 2 - 3 minutes to clear service line.

4. If right handed, hold sample bottle near bottom with right hand and remove closure and paper hood with left hand (reverse if left handed). DO NOT LAY CLOSURE DOWN. Hold in such a way to protect closure and bottle from contamination.

5. Allow slip of paper between closure and bottle neck to fall to floor.

6. Thrust bottle into flowing water and allow bottle to fill about 3/4ths full. DO NOT RINSE, especially if bottle contains sodium thiosulfate to neutralize chlorine in sample.

7. Carefully replace closure and hood and secure.

8. Label bottle and place on ice in ice chest for transportation to laboratory.

C. To sample river, strea, lake, etc.

1. Put on rubber gloves.

2. If right handed, hold sample bottle near bottom with right hand and remove closure and paper hood with left hand (reverse if left handed). DO NOT LAY CLOSURE DOWN. Hold in such a way to protect closure and bottle from contamination.

3. Allow paper strip between and bottle to fall to ground.

4. To fill sample bottle

a. Turn bottle neck opening down and plunge below surface of water quickly to prevent dechlorinating agent from running out.

b. Turn upward to face bottle opening into current to avoid contamination of water flowing into bottle with samplers hand.

c. Allow to fill to about 3/4 full. DO NOT OVERFILL especially if bottle contains a dechlorinating agent.

d. Lift quickly out of water and replace closure and hood.

5. Label bottle and place on ice in ice chest for transportation to laboratory.

III. COMMON ERRORS AND AFFECT ON RESULTS

A. No dechlorinating agent in bottle. Chlorine activity continues until sample tested so bacteria continue to die and coliform determination gives count which is lower than actual.

B. Sample not chilled when taken. Bacteria continue to multiply, so coliform determination gives count which is higher than actual.

C. Bottle or closure contaminated. Extra bacteria introduced, so coliform determination may give count which is higher than actual.

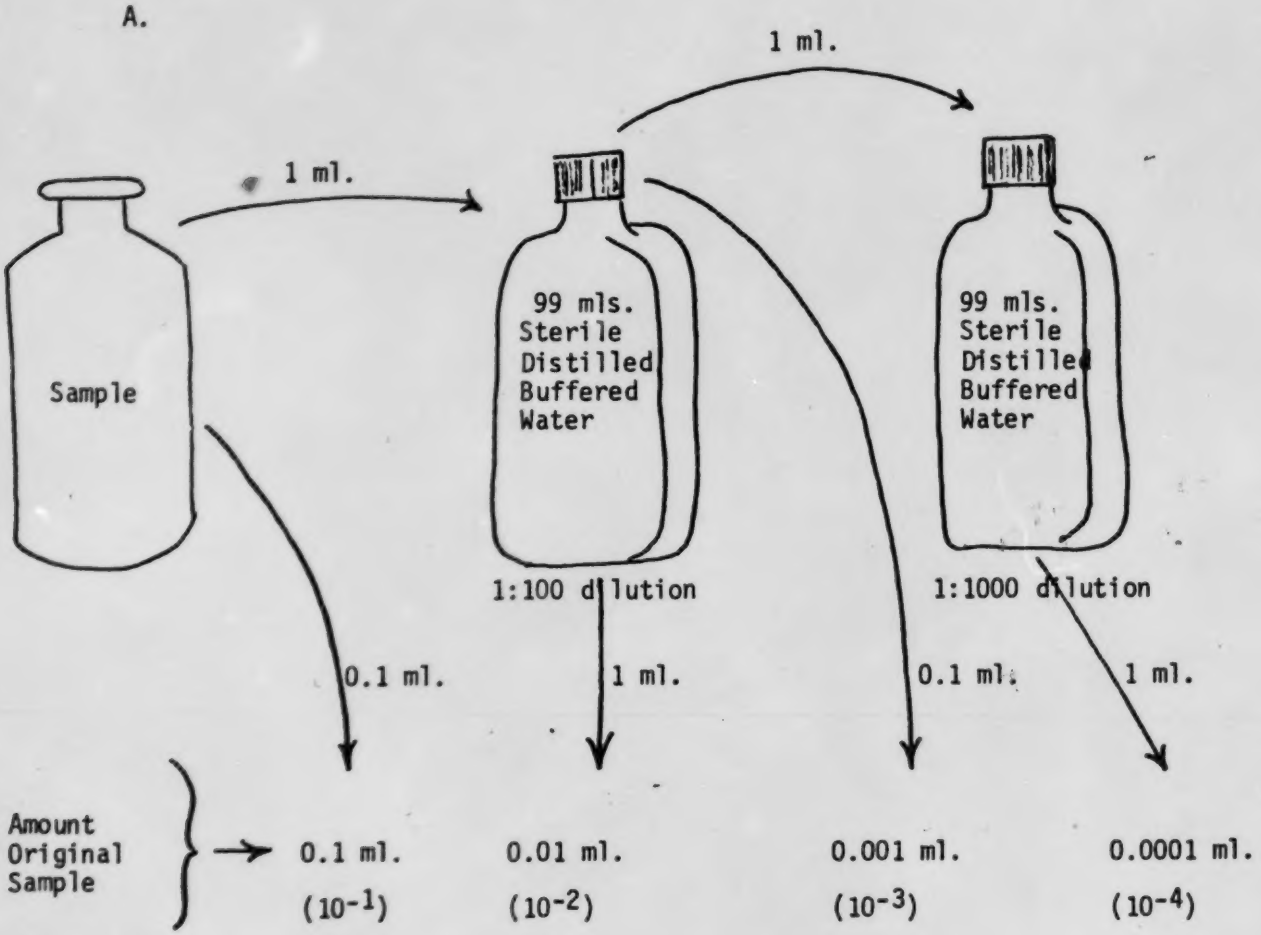
D. Sample not examined within 6 hrs. of collection. Bacteria will begin to die, so coliform determination will give counts which are lower than actual.

APPENDIX C - SAMPLE DILUTION

I. NECESSARY WHEN COUNT IS EXPECTED TO BE GREATER THAN 2,400 PER 100 ML.

II. PROCEDURE

A.



B. Place 0.1 ml. sample into culture tube for 0.1 ml. dilution.

C. For 0.01 ml. sample volume

1. Place 1 ml. sample into a 99 ml. dilution blank.
2. Shake vigorously 25 times in an arc of 12"
3. 1 ml. of this 1:100 dilution represents 0.01 ml. of original sample.

D. For 0.001 ml. sample volume deliver 0.1 ml. from 1:100 dilution into the culture tube.

E. For 0.0001 ml. sample volume

1. Place 1 ml. of the 1:100 dilution into a fresh 99 ml. dilution blank.

2. Shake vigorously 25 times in an arc of 12"

3. 1 ml. of this 1:10,000 dilution represents 0.0001 ml. original sample volume.

F. For 0.00001 ml. sample volume deliver 0.1 ml. from the 1:10,000 dilution into the culture tube.

III. PRECAUTIONS

A. All volume measurement must be accurate.

B. Any measurement error will be compounded in later steps.

C. Transfer sample volumes aseptically because any contamination will be carried through entire process.

HANDOUT B

TOTAL COLIFORM DETERMINATION IN DRINKING WATER

MEMBRANE FILTER TECHNIQUE

SECTION 1: INTRODUCTION TO COLIFORM TESTING

I. COLIFORMS ARE A GROUP OF BACTERIA

A. Need oxygen to survive

B. Rod-shaped

C. Gram negative

D. Ferment the sugar lactose with gas formation within 48 hrs. at 35° C.

E. Colonies produce a green metallic sheen on m-Endo media

G. See Figure #1 for size comparison

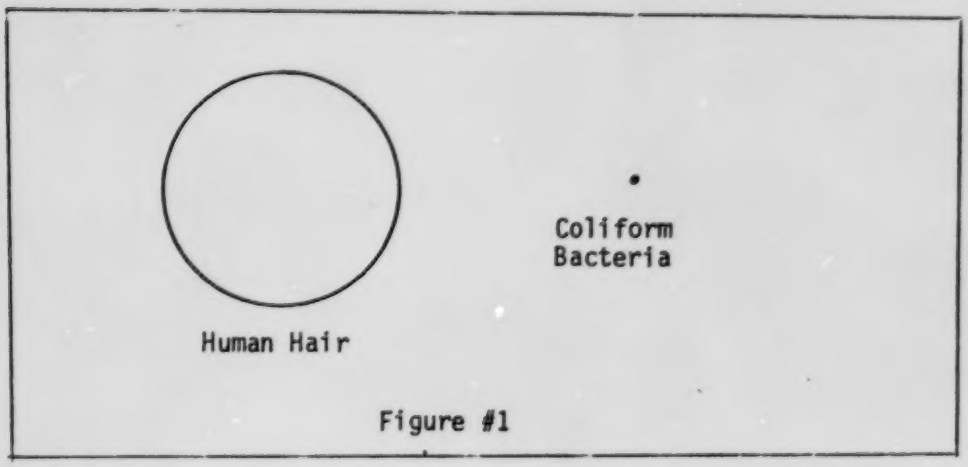


Figure #1

II. COLIFORMS IN DRINKING WATER MAY INDICATE

A. Fecal matter present therefore disease causing organisms present.

B. Insufficient chlorination therefore disease causing organisms still alive also.

III. WATER QUALITY STANDARDS FOR DRINKING WATER

A. Monthly average must not exceed 1 per 100 mls.

B. The coliform determination count must not exceed 4 per 100 mls. in:

1. 2 consecutive samples

2. More than 1 sample per month if fewer than 20 samples per month are examined.

3. In more than 5% of samples if more than 20 samples per month are examined.

C. Number of samples which must be taken monthly.

Population served:	Minimum number of samples per month
25 to 2,500	2
2,501 to 3,300	3
3,301 to 4,101	4
4,100 to 4,900	5
4,901 to 5,800	6
5,801 to 6,700	7
6,701 to 7,600	8
7,601 to 8,500	9
8,501 to 9,400	10
9,401 to 10,300	11
10,301 to 11,100	12
11,101 to 12,000	13
12,001 to 12,900	14
12,901 to 13,700	15
13,701 to 14,600	16
14,601 to 15,500	17
15,501 to 16,300	18
16,301 to 17,200	19
17,201 to 18,100	20
18,101 to 18,900	21
18,901 to 19,800	22
19,801 to 20,700	23
20,701 to 21,500	24
21,501 to 22,300	25
22,301 to 23,200	26
23,201 to 24,000	27
24,001 to 24,900	28
24,901 to 25,000	29
25,001 to 28,000	30
28,001 to 33,000	35
33,001 to 37,000	40
37,001 to 41,000	45
41,001 to 46,000	50
46,001 to 50,000	55
50,001 to 54,000	60
54,001 to 59,000	65
59,001 to 64,000	70
64,001 to 70,000	75
70,001 to 76,000	80
76,001 to 83,000	85
83,001 to 90,000	90
90,001 to 96,000	95
96,001 to 111,000	100
111,001 to 130,000	110
130,001 to 160,000	120
160,001 to 190,000	130
190,001 to 220,000	140
220,001 to 250,000	150
250,001 to 290,000	160
290,001 to 320,000	170
320,001 to 360,000	180

SECTION 2: EQUIPMENT AND MEKIA PREPARATION

I. LIST OF BENCH EQUIPMENT MEDIA AND REAGENTS

A. Bench equipment

1. Hot plate
2. Balance with 0.5 gm. sensitivity
3. pH meter
4. Steriomicroscope (or other 10 x magnification device)
5. Round tipped forceps
6. Burner with open flame
7. Pipet soaking jar
8. Vacuum source

B. Glassware

1. 250 ml screw cap erlynmeyer flasks
2. Sample bottles
3. 100 ml. graduated cylinders
4. Filtering flasks
5. Membrane filter funnel
6. Reagent bottles
7. 4 oz. ointment jars
8. Re-pipettor with erlynmeyer flask
9. 100 ml. dilution bottles

C. Expendables

1. 10 ml. pipets
   a. Sterile, disposable cotton plugged, individually wrapped
   b. Or reusable with pipet can (to sterilize in)

2. 1 ml. pipets
   a. Sterile, disposable cotton plugged, individually wrapped
   b. Or reusable with pipet can (to sterilize in)
3. Membrane Filters
   a. 0.45 m pore rating
   b. 47 mm diameter
   c. White and gridded
   d. Sterile
4. Adsorbent pads
   a. High quality filter paper
   b. 48 mm in diameter
   c. Able to absorb 1.8 - 2.2 ml. of broth growth media
   d. Sterile
5. 50 x 12 mm sterile petri dishes with tight fitting covers
6. Non-adsorbant cotton
7. Cotton gauze
8. Brown Kraft wrapping paper
9. Aluminum foil
10. Rubber gloves
11. Paper towels
12. Sponge

D. Safety equipment
   1. Fire extinguisher
   2. Fire blanket
   3. First aid kit
   4. Emergency shower
   5. Emergency eye wash

E. Reagents and media

1. Reagent grade 95% ethanol
2. m-Endo broth or m-Endo agar
3. Disinfectant
4. Peptone or $KH_2 PO_4$
5. 1 N NaOH

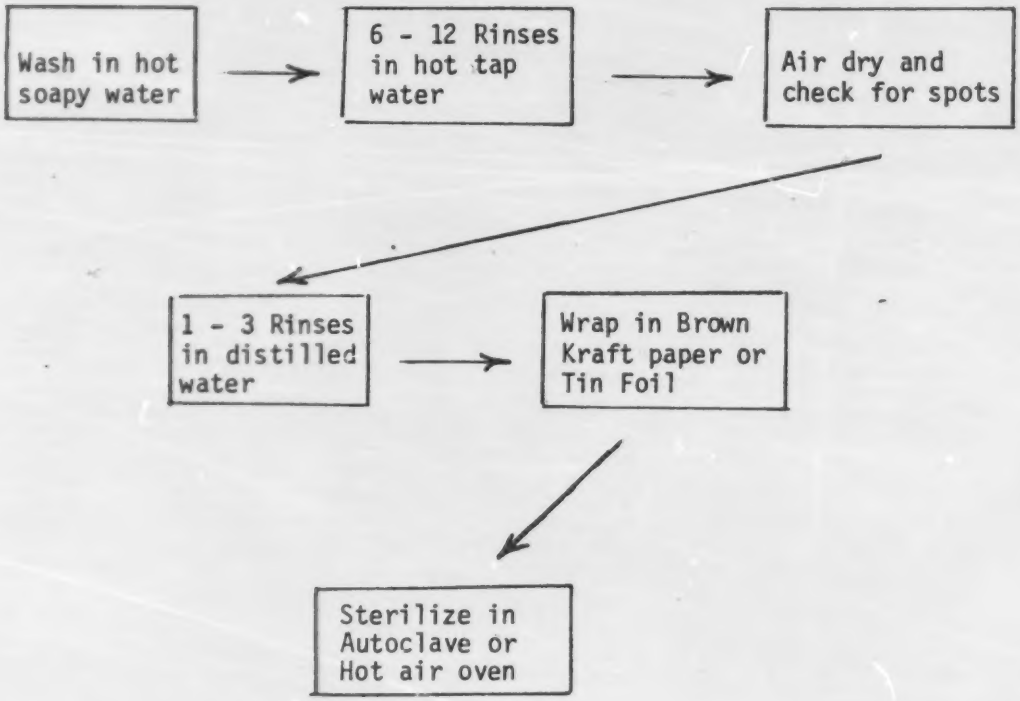
II. BENCH EQUIPMENT PREPARATION & FUNCTION

A. pH meter: Used to check pH of prepared media and reagents.

B. Stereomicroscope - 10 x 15 x: Used to count coliform colonies on membrane filters.

C. Balance with 0.5 gm sensitivity at 150 gms. to weigh media and reagents.

D. Filtration Equipment & Glassware Preparation


Wash in hot soapy water
6 - 12 Rinses in hot tap water
Air dry and check for spots
1 - 3 Rinses in distilled water
Wrap in Brown Kraft paper or Tin Foil
Sterilize in Autoclave or Hot air oven

E. Function of Filtration Equipment & Glassware

1. Vacuum pump and tubing which must be able to pull 22" vacuum.
2. Trap flask which acts as safety trap to keep water out of pump.
3. Filtering flask
   a. Traps water after it passes through filter.
   b. Must be sterilized as often as filtering funnel.
4. Filtering Funnel
   a. Must seal membrane in place with no leaks.
   b. Available in stainless steel, corocillicate glass or autoclavable plastic.
   c. Need not be sterilized between consecutive filtrations.
   d. Must be sterilized if more than 1 hr. has elapsed since last sample filtration.
5. Round tipped forceps
   a. Use to handle membrane
   b. Must be free from rough or sharp edges
6. Sterile rinse bottle
   a. Filled with sterile distilled buffered rinse water
   b. Used to rinse inner surfaces of funnel between consecutive filtrations.
   c. Glass erlynmeyer with repipettor
7. A 100 ml. graduated cylinder for each water sample
   a. Used to measure water samples
   b. Must be sterile
   c. Must have 1 for each sample
8. Burner with open flame to ignite alcohol
   a. Bunsen burner
   b. Alcohol burner

F. Function of Expendable Equipment

1. Sterile 0.45 m membrane filters for water testing for trapping bacteria.
2. Sterile adsorbant pads for holding growth media.
3. Sterile 50 x 12 mm petri dishes for culturing inoculated membrane.
4. Sterile 1 ml. and 10 ml. pipets for measuring water samples.

III. REAGENT AND MEDIA PREPARATION

A. Use only distilled water

B. Sterile distilled buffered water - 2 types

1. Phosphate buffered water

a. Stock solution

1. Dissolve 34 gms. $KH_2 PO_4$ in 500 mls. distilled water in volumetric flask.
2. Adjust pH to 7.2 with 1 N NaOH
3. Dilute 5o 1 1. with distilled water

b. To make buffered water

1. Add 1.25 mls. stock to 1 1. distilled water
2. Mix, dispense and sterilize 20 min. at 121^{o} C. (15 psi)

2. Peptone dillution water

a. Stock solution

1. Dissolve 10 gms. peptone in 100 mls. water
2. To store, sterilize 15 min. 121^{o} C. and store in refrigerator
3. Discard if it becomes cloudy.

b. To make dilution water

1. Add 1 ml. stock solution per 100 mls. distilled water.
2. Mix, dispense, sterilize 20 min. at 121^{o} C. (15 psi)

3. Sterilization and uses of distilled buffered water

   a. Rinsing funnels between samples

      1. Dispense and sterilze in autoclave 20 min. at 121° C. in cotton stoppered autoclavable rinse bottles.

      2. Use slow exhaust.

      3. Do not fill bottle over 3/4 full.

      4. Sterilze delivery tube separately and aseptically assemble.

   b. Dilution of samples

      1. Dispense 99 mls. plus 4 mls. to allow for evaporation in 99 ml. dilution blanks.

      2. Sterilze in autoclave 20 min. 15 121° C (15 psi)

      3. Use slow exhaust.

      4. Sterilze with caps loose.

      5. Tighten caps when removed from autoclave.

C. Sodium Thiosulfate Solution

   1. Stock solution

      a. Weigh 10 gms. of sodium thiosulfate

      b. Dissolve in 50 - 60 mls. distilled water in a 100 ml. volumetric flask.

      c. Add distilled water to bring to a final volume of 100 mls.

      d. Transfer to stoppered, 100 ml. labeled bottle and store in refrigerator.

   2. For use transfer 0.1 ml. stock solution (for each 4 oz. volume) to sample bottle before sterilization.

D. m-Endo Preparation

   1. Order in amounts to fit needs

      a. Dehydrated broth media

         1. 1 lb. bottle will make enough media for 4,000 filtrations.

         2. ½ lb. bottle will make enough media for 1,000 filtrations.

      b. Ampoules of prepared broth media

         1. Can be ordered - 24 per package

         2. Must be refrigerated and used within 1 year.

2. Prepare dehydrated m-Endo media the day it is to be used.
3. Prepare dehydrated m-Endo media according to manufacturers instruction.
   a. Do not overheat
   b. Do not sterilize
   c. Protect from light while cooling to room temperature
   d. Dispense when cool and use immediately.

SECTION 3: MEMBRANE FILTRATION PROCEDURE

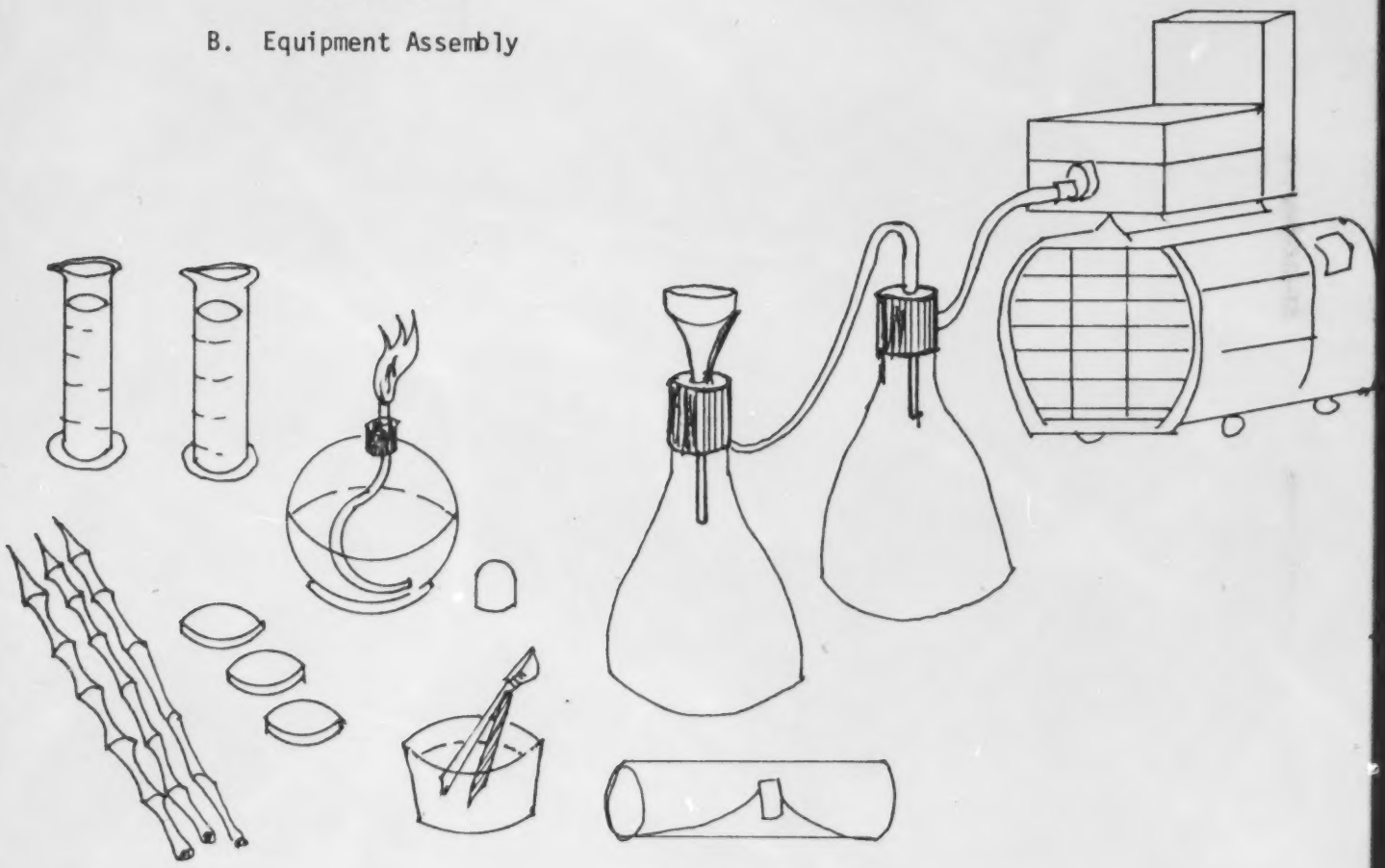
I. DATA SHEET PREPARATION

II. WORK AREA PREPARATION

A. Wash hands and disinfect work bench top

1. Lowers possibility of sample contamination leading to duplication of work.

B. Equipment Assembly



1. Connect vacuum tubing pump to trap flask.
2. Connect vacuum tubing trap flask to filtering flask.
3. Aseptically seal funnel base in vacuum flask.
4. Lay wrapped funnel in front.

5. Lay out burner forceps, alcohol jar, sterile M.F.'s, sterile graduates, sterile pipets.

C. Dispense pads into 15 x 12 mm. Petri dishes

1. From pack use flamed forceps.

2. From 100 pack use dispenser.

D. Dispense broth media

1. Use sterile 10 ml. pipet.

2. Dispense 1.8 to 2.2 ml. onto each pad.

3. Immediately before use decant excess media by gently tipping dish.

III. SAMPLE FILTRATION

A. Place membrane filter onto funnel base grid side up.

1. Membrane acts as trap for bacteria.

2. Membrane acts as support for colony growth.

B. Replace funnel top

C. Add sample and filter

1. If greater than 20 mls. just pour in.

2. If less than 20 mls. first pour in 20 mls. sterile distilled buffered water, then add sample volume to this.

3. Filter completely at 22" vacuum.

4. Rinse inner surfaces of funnel.

a. 3 separate rinses 20 mls. each.

b. Use sterile distilled buffered water

c. Allow each rinse to filter completely before adding next.

d. This procedure rinses bacteria from inner surfaces of funnel.

1. Makes sterilization between consecutive samples unnecessary.

2. If more than 1 hr. elapses between samples re-sterilize unit.

D. Remove filter from unit

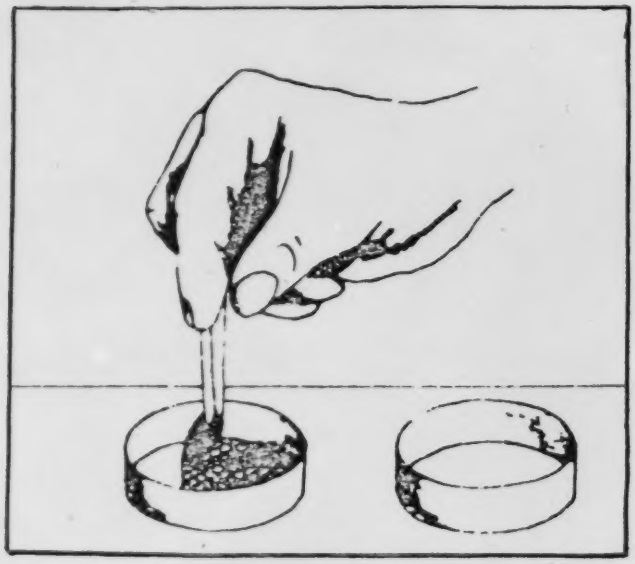
1. Carefully remove funnel top without disrupting membrane.

2. Dip forcep tips into alcohol and ignite to sterilze.

3. Pick up membrane with forceps touching only outer 1/8" inch of membrane.

IV. CULTURING MEMBRANE

A. Place membrane on saturated pad



1. Roll membrane to prevent air being trapped under membrane.

2. If air is trapped re-roll membrane.

3. Do not remove air by "smoothing with forceps".

4. Replace dish cover

B. Incubate cultured membrane

1. Invert dish

a. Membrane facing down

b. Keeps moisture in pad

c. Keeps moisture from dripping from lid onto membrane surface.

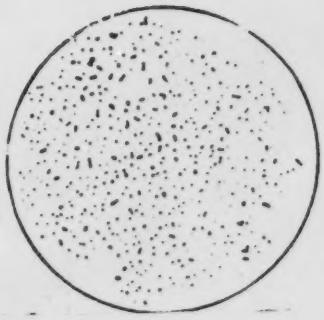
2. Incubate in 35^{0} incubator for 22 - 24 hours.

a. Keep incubator humidified.

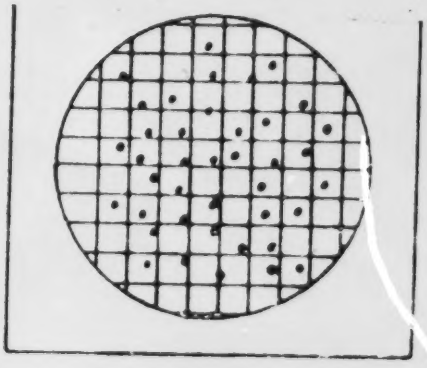
b. Allows bacteria to multiply and form colonies.

SECTION 4: MEMBRANE FILTER COUNTING PROCEDURE

I. COUNTING RANGE



CHOOSE THE CORRECT MEMBRANE

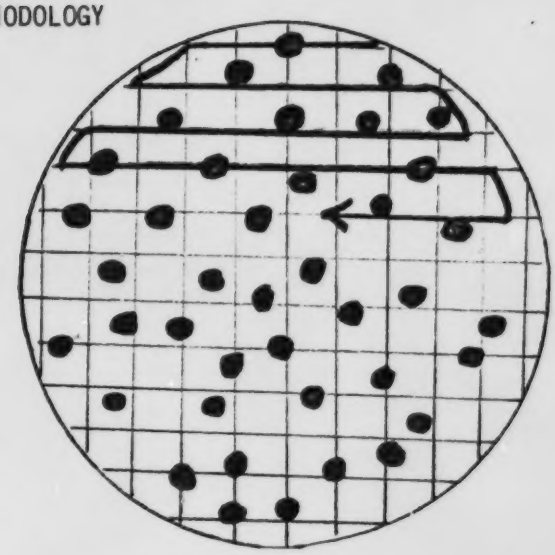


A. 20 - 80 coliform colonies

B. No more than 200 colonies total

C. Drinking water will give less than 20 coliform colonies

II. COUNTING METHODOLOGY



A. Count colonies with the aid of the grid lines.

B. Count in a back and forth motion.

1. Count those colonies touching the top line.

2. Do not count those colonies touching the bottom line.

III. COLONY DIFFERENTIATION

A. Coliform colonies

1. Dark red

2. Green metallic surface sheen

B. Non-coliform colonies

1. White to dark in color.

2. No metallic surface sheen.

IV. ATYPICAL COLONIES

A. Can be confirmed

B. Follow method prescribed in "Standard Methods for the Examination of Water and Wastewater".

SECTION 5: PROCESSING USED GLASSWARE

I. CONTAMINATED BUT UNCULTURED GLASSWARE

A. Sterilization unnecessary

B. Empty contents down drain

C. Wash, rinse, dry as previously described

D. Prepare for next testing series

II. GLASSWARE CONTAINING CULTURES

A. Sterilize in an autoclave

B. Empty contents down drain or in garbage

C. Wash, rinse and dry as previously described

D. Prepare for next testing series

III. DISPOSABLES

A. Discard in polypropylene bag

B. Sterilze in autoclave

C. Dispose of in garbage

SECTION 6: DATA INTERPRETATION AND EVALUATION

I. CALCULATION OF COUNT PER 100 ML.

Sample A		Sample B		Sample C	
Amt. Filtered	Count	Amt. Filtered	Count	Amt. Filtered	Count
100 mls.	64	100 mls.	107	100 mls.	4
50 mls.	26	50 mls.	53	10 mls.	0
10 mls.	4	10 mls.	14	1 mls.	0
$\frac{\text{Count + count}}{\text{Total amt. filtered}} \times 100$		$\frac{\text{Count}}{\text{Amt. filtered}} \times 100$			
Example:		Example:			
$\frac{64}{100} + \frac{26}{50} = \frac{90}{150} \times 100 = 60$		$\frac{53}{50} \times 100 = 106$			
Report as: 60/100 ml.		Report as: 110/100 ml		Report as: 4/100 ml.	

A. From Above Figure

1. Sample A - 2 counts within accepted range.
2. Sample B - 1 count within accepted range.
3. Sample C - Counts too low on all dilutions.

B. Counts too high on all dilution.

1. Report as TNTC (Too numerous to count)
2. Request new sample

C. Report all counts to 2 significant figures only.

1. i.e. report 392/100 ml. as 390/100 ml.

II. DATA EVALUATION

A. Drinking water standards

1. Monthly average must not exceed 1 per 100 ml.
2. The coliform determination count must not exceed 4 per 100 mls. in:
   a. 2 consecutive samples

b. More than 1 sample per month if fewer 20 samples per month are examined.

c. More than 5% samples if more than 20 samples per month are examined.

APPENDIX A - LABORATORY PREPARATION

I. SETTING LABORATORY RULES

A. Dress Code

1. Must wear lab coat or apron at all times.
2. Shoes must have full foot protection.
3. Long hair must be tied back.
4. Must wear protective clothing where applicable.
   a. Goggles or safety glasses
   b. Asbestos gloves

B. Safety Equipment

1. General Equipment
   a. Fire extinguisher
   b. Fire blanket
   c. First aid kit
   d. Emergency shower
   e. Emergency eye wash
2. Personal equipment for each employee
   a. Lab coat or apron
   b. Goggles
   c. Asbestos gloves
3. Safety rules
   a. Must be set and enforced by supervisor
   b. All accidents must be reported to supervisor

C. Record Keeping

1. Must be maintained at all times.
2. Should include all:

a. Purchase records

b. Equipment specifications, warranties, maintenance and instruction manuals.

c. Accident reports

d. Testing data

e. Pertinent communications

f. Employee records

II. LABORATORY CLEANLINESS

A. Types of disinfectants

1. 70% Ethanol

2. Phenols i.e. O-Syl

3. Quaterniary ammonium compounds

4. Halogen compounds

5. Activated sialdehyde i.e. cidex

B. Use of disinfectants

1. Weekly

a. Wipe down all shelves removing all glassware and books.

b. Wipe down all incubators, inside and outside.

c. Wipe out inside of autoclave.

2. Daily

a. Wipe down tops of all counters, large pieces of equipment

3. Immediately before testing disinfect work area

4. Immediately disinfect spills

C. Sources of Contamination

1. Dirt around lab

2. Spilled samples or cultures

3. Un-autoclaved bacterial garbage

4. Chemical contamination from use of glassware for both Chemistry testing and Bacterial testing.

III. GLASSWARE WASHING

A. All glassware must be thoroughly washed in non-toxic detergent

1. i.e. Alconox

2. Removes bacterial scum from glassware

B. Rinse 6 - 12 times in hot tap water

1. Removes detergent residue

2. Residue is harmful to bacteria

C. Final rinse 1 - 3 times in distilled water

1. Removes mineral residue from tap water

2. Prevents water spotting

D. Air Dry

1. Any spot indicates dirt

2. Rewash before using

IV. PACKAGING EQUIPMENT AND STERILIZATION

A. Reasons for packaging

1. Creates a bacteria barrier

2. Allows for storage of sterile equipment

B. Proper labeling

1. Define contents

2. Date to aid in equipment rotation

C. Sterilization of equipment - 2 Acceptable Methods

1. Autoclave

a. All rubber, metal and glassware and some plastics

b. Normal cycle 15 min. 15 121^{o} C.

c. Exhaust rapidly

2. Hot air sterilizing oven

a. Dry glassware and metal objects only

b. Normal cycle 1 hr. at 170^{o} C.

c. Allow to cool before use

d. Package pipets in metal containers

e. Package other equipment with aluminum foil

V. MAJOR LABORATORY EQUIPMENT

A. Autoclave

1. Before using read and follow manufacturers installation use and maintenance instructions and safety precautions.

2. Normal sterilization = 15 psi yielding 121° C. for 15 min.

3. Use to sterilize liquids and non-heat sensitive equipment.

a. Most plastics are not autoclavable and sterilized by manufacturer.

b. Sterilized media and reagents must be removed from autoclave as soon as possible after autoclave is opened.

c. Glassware may be sterilized in autoclave but must be allowed to dry before removing from autoclave.

B. Hot air sterilizing oven

1. Before using read and follow manufacturers installation, use, and maintenance instructions and safety precautions.

2. Normal Sterilization = 1 hour at 180° C.

3. Use to sterilize glass and metal only.

a. Rubber and plastics will melt

b. Liquids will evaporate and grow media components will be destroyed

C. 35° Incubator

1. Before using read and follow manufacturers installation and maintenance instructions and safety precautions.

2. Place in permanent location

a. Out of drafts and direct sunlight

b. Convenient to laboratory bench and electrical outlet

3. Install thermometer

a. NBS (National Bureau of Standards) certified thermometer

b. Mercury bulb of thermometer should be suspended in bottle filled with water.

c. Locate centrally in incubator.

4. Install shallow pan of water in bottom of incubator.

   a. Maintains condition of saturated relative humidity required in bacteriological incubator.

   b. Check daily and fill as necessary to keep water in pan at all times.

5. Adjust temp. to $35^{o} \pm 0.5^{o}$ C.

   a. Follow manufacturers instructions

   b. Allow 1 hr. between temperature adjustments.

   c. Record temp. of incubator daily.

D. Water Distillation and Deionizing Unit

1. Before using, read and follow manufacturers installation, use and maintenance instructions and safety precautions.

2. Produces reagent grade water for use in making reagents and media and rinsing glassware.

E. Refrigerator

1. Set to maintain a 4^{o} C. temperature.

2. Use to hold samples waiting to be tested and to store some prepared media and reagents.

F. Glassware washer

1. Before using, read and follow manufacturers installation, use and maintenance instructions and safety precautions.

2. Automatically washes and rinses glassware.

3. Do not use home dishwasher as it does not have proper plumbing

APPENDIX B - COLLECTING SAMPLES FOR BACTERIOLOGICAL EXAMINATION

I. EQUIPMENT PREPARATION

A. Sample bottles must be:

1. At least 100 ml. capacity with a large neck opening.

2. Thoroughly cleaned with detergent, rinsed 6 times in hot tap water, rinsed finally in distilled-deionized water, then air dried.

3. Free from spots, scum, chips, cracks, excessive scratches and other damage on which bacteria may lodge.

4. Closed with preferably an all glass ground cap closure (but screw caps can be used providing liners are free from contamination and provide a non-leaking seal.

5. Sterilized in an autoclave at 121° C. for 15 min. with Kraft paper or tin foil hood covering caps and necks of bottles and slip of paper between bottleneck and glass stopper to prevent glass stopper from sticking.

B. Bottles intended for use in collection of chlorinated samples must have a 10% sodium thiosulfate solution added at the rate of 0.1 ml. for each 4 oz. bottle prior to sterilization and sterilized in bottle.

C. Labels must be:

1. Clean and unused.

2. Attached to bottle by a means not affected by water (i.e. string or wire.)

D. Label markers must be:

1. Permanent type not affected by water.

2. Able to mark on label.

E. Sampling devices must be in working condition and properly maintained.

F. Germicide must be available to clean up spills but must not come in contact with sample or any equipment touched by sample.

G. Rubber gloves must fit and not be punctured.

H. Ice chest for transporting sample must be:

1. Sufficient size to accommodate all samples.

2. Undamaged with tight cover so cold temperature can be maintained inside.

3. Filled with enough ice to quickly chill sample but little or no free water.

I. Refrigerator must be set at 2 - 10° C. and used if samples are not examined upon immediate return to lab.

II. SAMPLE COLLECTION

A. To take sample from spigot or tap:

1. Find spigot with direct main connection

2. Put on rubber gloves.

3. Flush spigot at full flow for 2 - 3 min. to clear service line

4. If right handed, hold sample bottle near bottom with right hand and remove closure and paper hood with left hand (reverse if left handed). DO NOT LAY CLOSURE DOWN. Hold in such a way to protect closure and bottle from contamination.

5. Allow slip of paper between closure and bottle neck to fall to floor.

6. Thrust bottle into flowing water and allow bottle to fill about 3/4ths full. DO NOT RINSE, especially if bottle contains sodium thiosulfate to neutralize chlorine in sample.

7. Carefully replace closure and hood and secure.

8. Label bottle and place on ice in ice chest for transportation to laboratory.

C. To sample river, stream, lake, etc.

1. Put on rubber gloves.

2. If right handed, hold sample bottle near bottom with right hand and remove closure and paper hood with left hand (reverse if left handed). DO NOT LAY CLOSURE DOWN. Hold in such a way to protect closure and bottle from contamination.

3. Allow paper strip between and bottle to fall to ground.

4. To fill sample bottle

a. Turn bottle neck opening down and plunge below surface of water quickly to prevent dechlorinating agent from running out.

b. Turn upward to face bottle opening into current to avoid contamination of water flowing into bottle with samplers hand.

c. Allow to fill to about 3/4 full. DO NOT OVERFILL especially if bottle contains a dechlorinating agent.

d. Lift quickly out of water and replace closure and hood.

5. Label bottle and place on ice in ice chest for transportation to laboratory.

III. COMMON ERRORS AND AFFECT ON RESULTS

A. No dechlorinating agent in bottle. Chlorine activity continues until sample tested so bacteria continue to die and coliform determination gives count which is lower than actual.

B. Sample not chilled when taken. Bacteria continue to multiply, so coliform determination gives count which is higher than actual.

C. Bottle or closure contaminated. Extra bacteria introduced, so coliform determination may give count which is higher than actual.

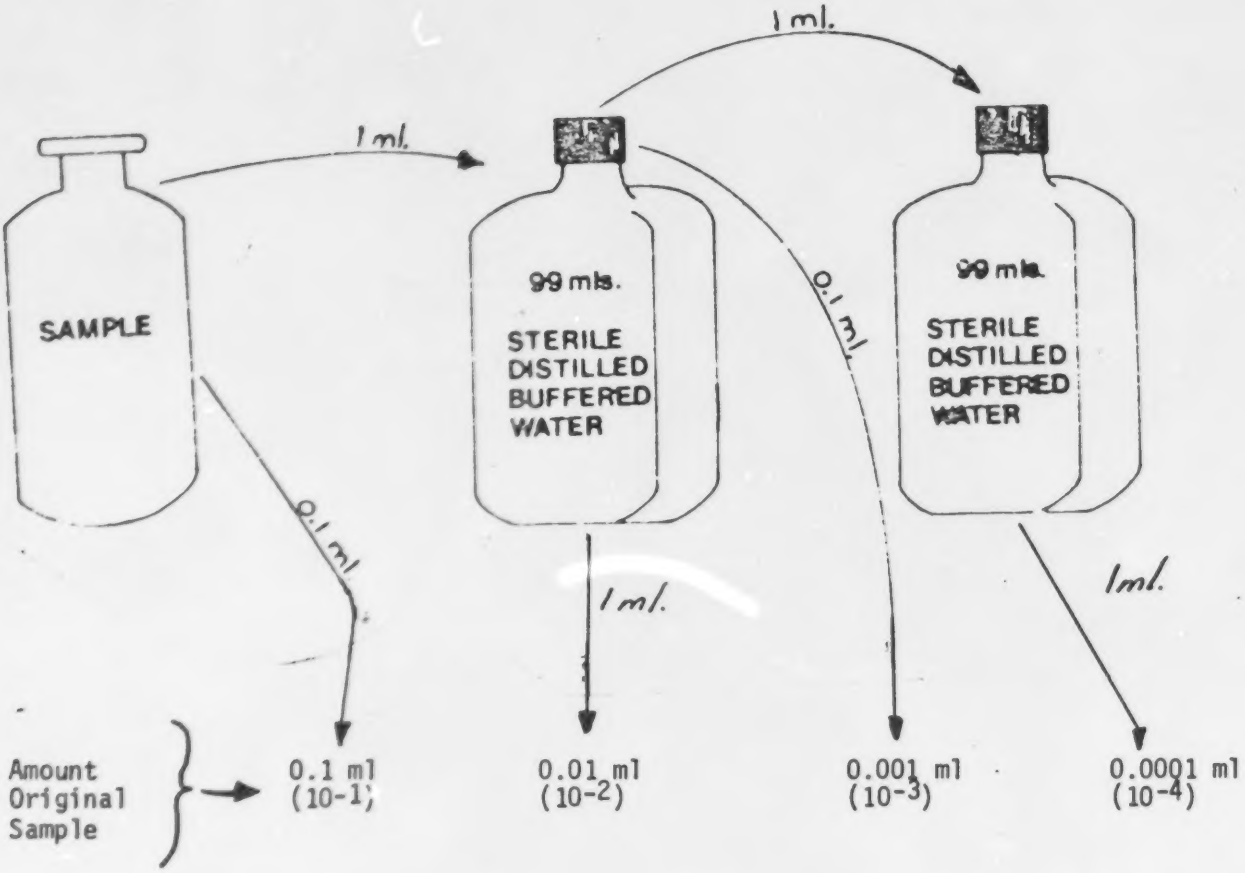
D. Sample not examined within 6 hrs. of collection. Bacteria will begin to die, so coliform determination will give counts which are lower than actual.

APPENDIX C - SAMPLE DILUTION

I. NECESSARY WHEN COUNT IS EXPECTED TO BE GREATER THAN 8000 PER 100 ML

II. PROCEDURE

A.



B. Place 0.1 ml. sample into funnel for 0.1 ml. dilution.

C. For 0.01 ml. sample volume

1. Place 1 ml. sample into a 99 ml. dilution blank.
2. Shake vigorously 25 times in an arc of 12"
3. 1 ml. of this 1:100 dilution represents 0.01 ml. of original sample.

D. For 0.001 ml. sample volume deliver 0.1 ml. from 1:100 dilution into funnel.

E. For 0.0001 ml. sample volume

1. Place 1 ml. of the 1:100 dilution into a fresh 99 ml. dilution blank.
2. Shake vigorously 25 times in an arc of 12"
3. 1 ml. of this 1:10,000 dilution represents 0.0001 ml. original sample volume.

F. For 0.00001 ml. sample volume deliver 0.1 ml. from the 1:10,000 dilution into funnel.

III. PRECAUTIONS

A. All volume measurement must be accurate.

B. Any measurement error will be compounded in later steps.

C. Transfer sample volumes aseptically because any contamination will be carried through entire process.

TOTAL COLIFORM DETERMINATION IN DRINKING WATER

Transparancy List

Transparancy #1: Sample dilution

Transparancy #2: MPN equipment

Transparancy #3: Pipet and loop

Transparancy #4: Positive test

Transparancy #5: Recording MPN test data

Transparancy #6: MPN chart

Transparancy #7: MF equipment

Transparancy #8: MF equipment set up

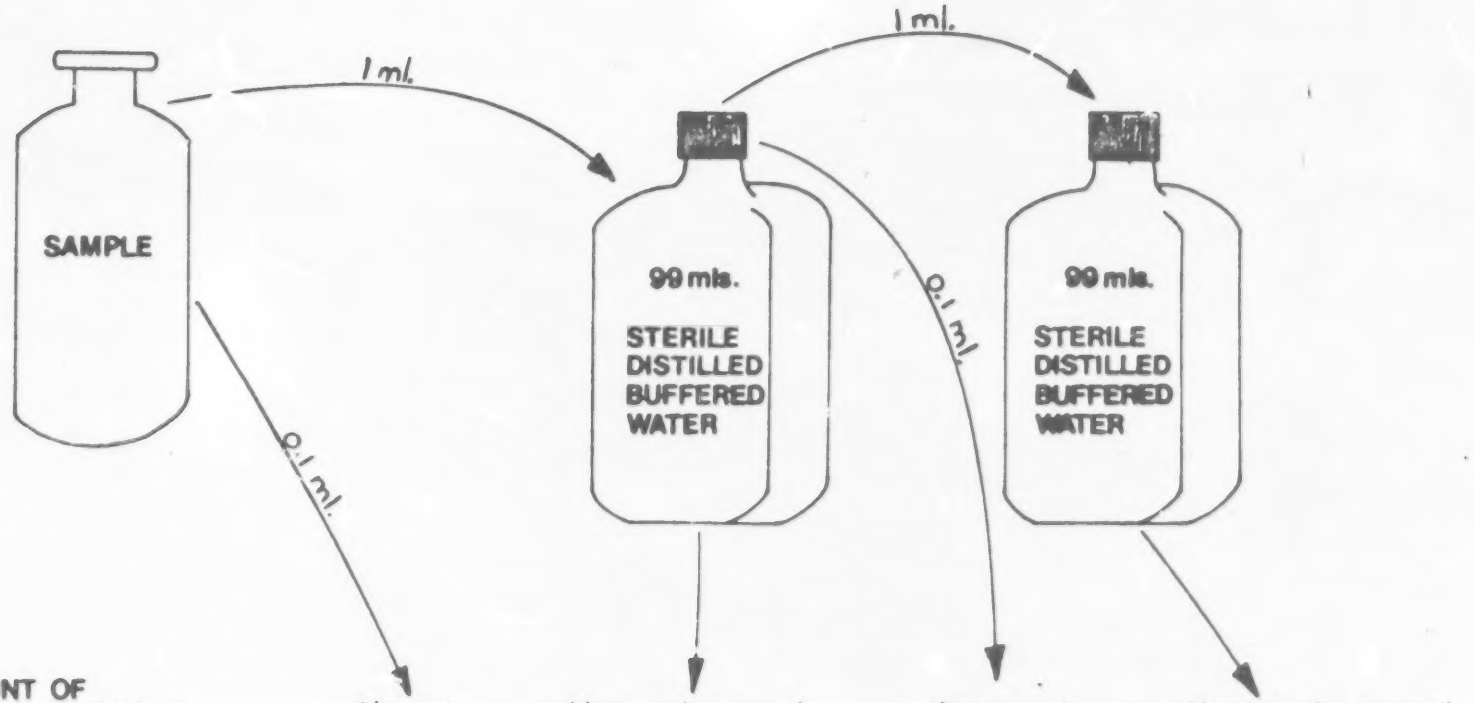
Transparancy #9: Plating method

Transparancy #10: Choose correct MF to count

Transparancy #11: Counting methodology

Transparancy #12: Calculating count per 100 mls.

SAMPLE DILUTION

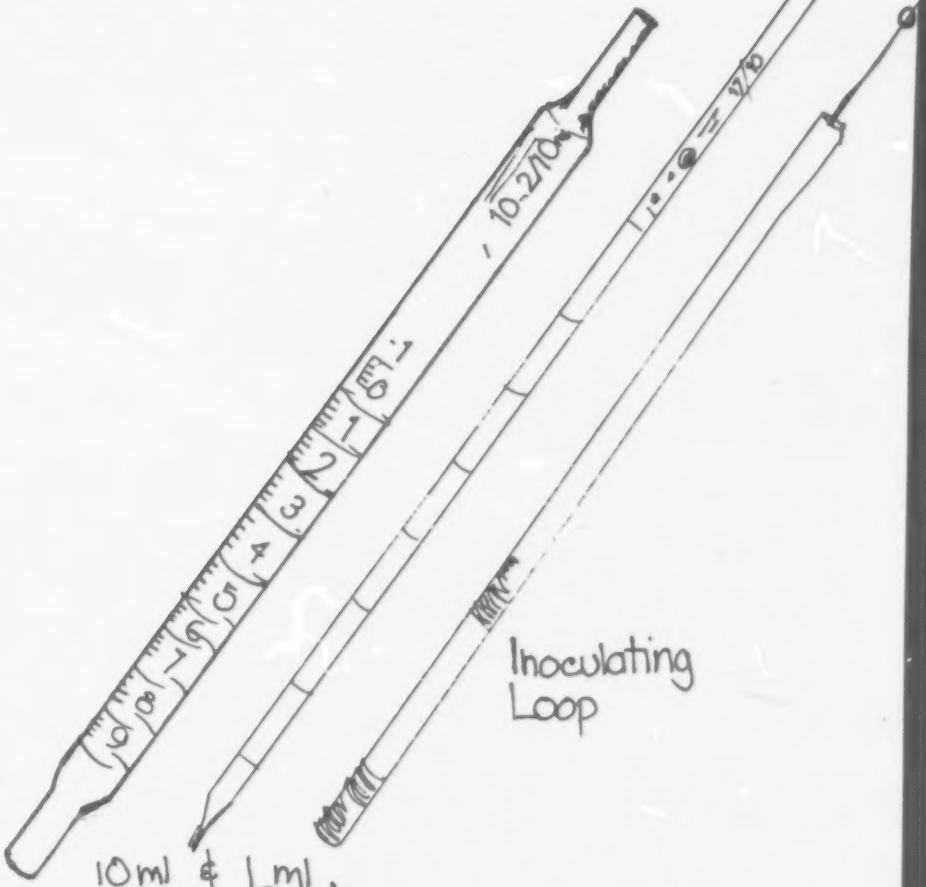


Multiple Tube Technique Equipment

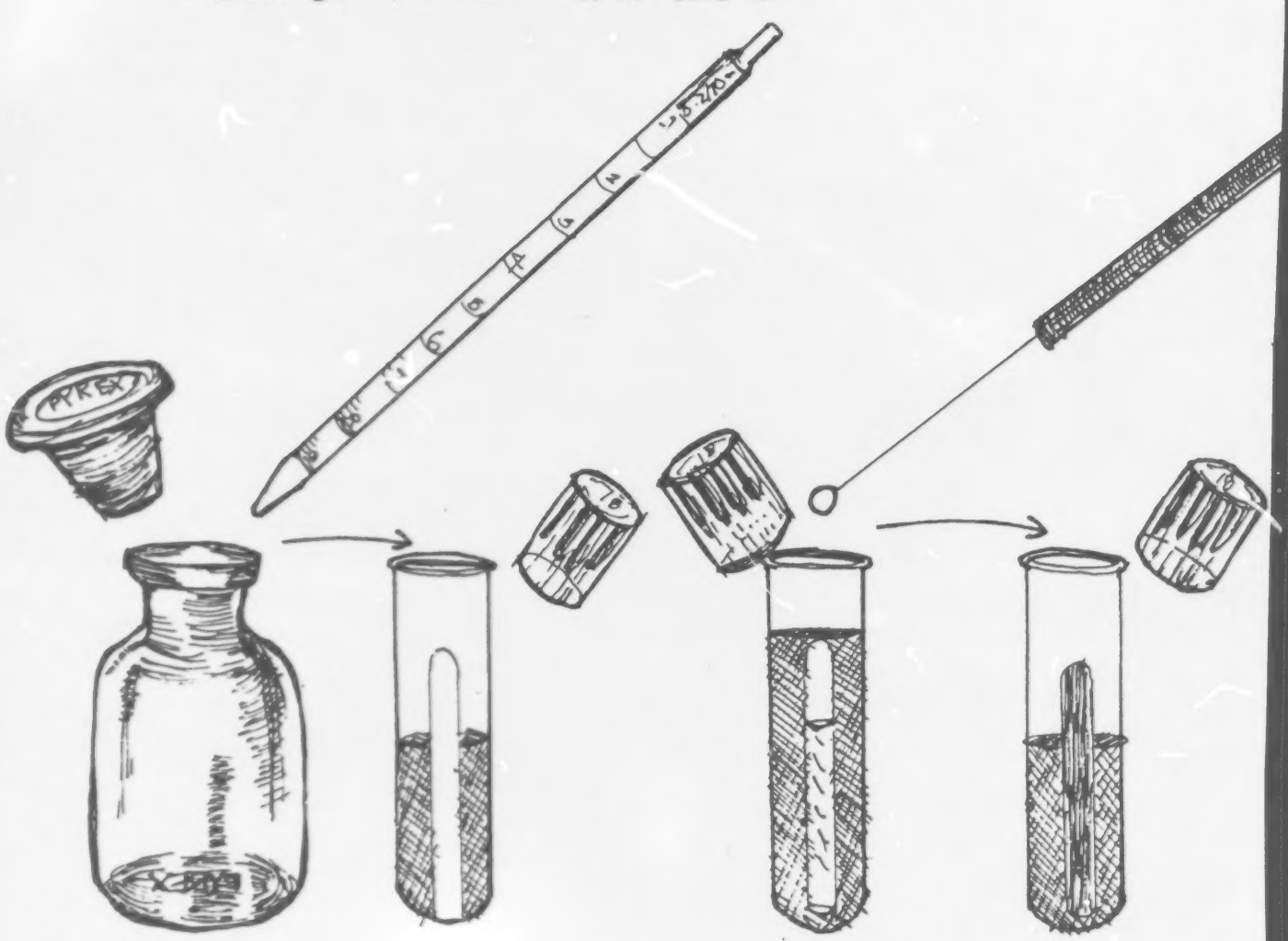
18x 150 mm

6x50 mm

Test Tubes


1.0
1.0
10-2/10
1.0
1.0
1.0

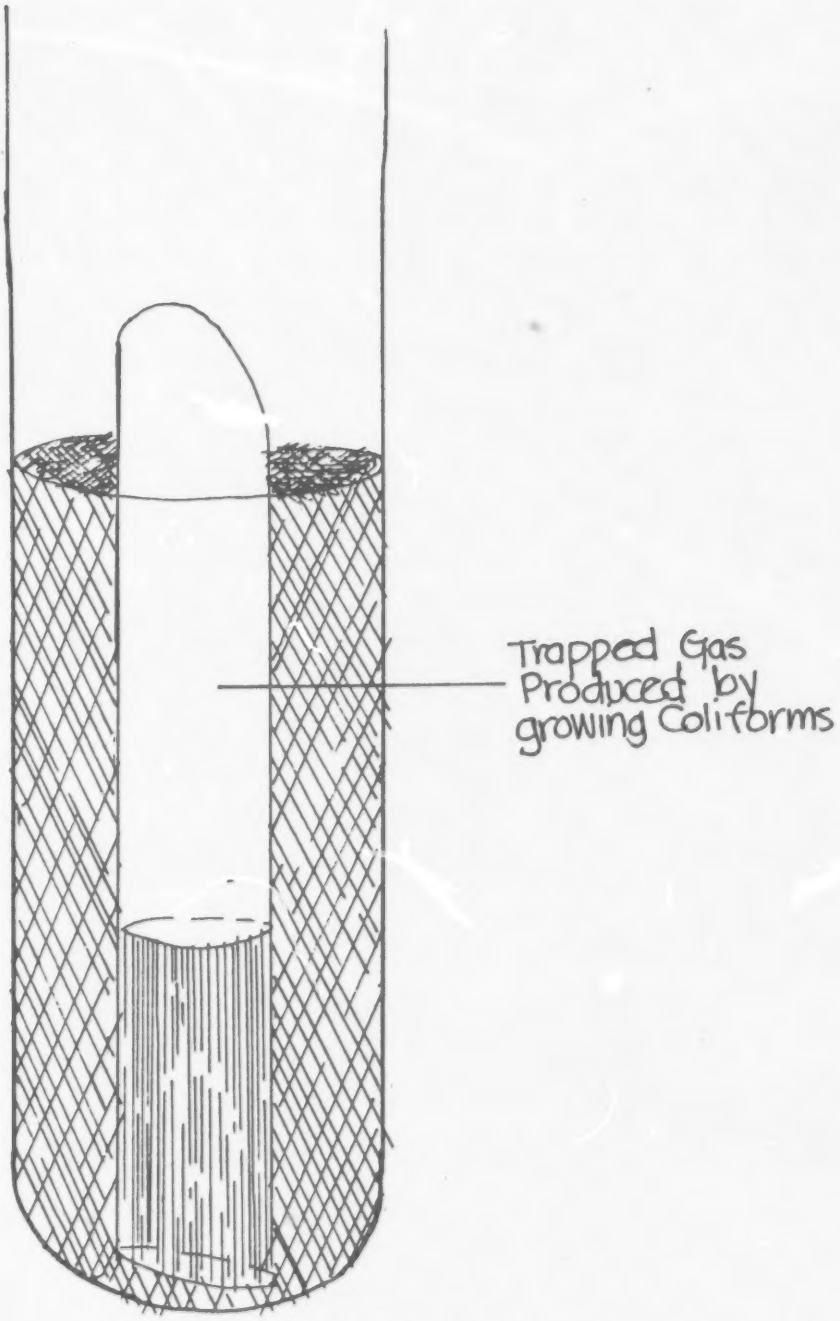
Use of PIPET and LOOP



Use Pipet to inoculate presumptive media with sample.

Use loop to transfer growth from positive presumptive media to confirmed media.

Positive Test

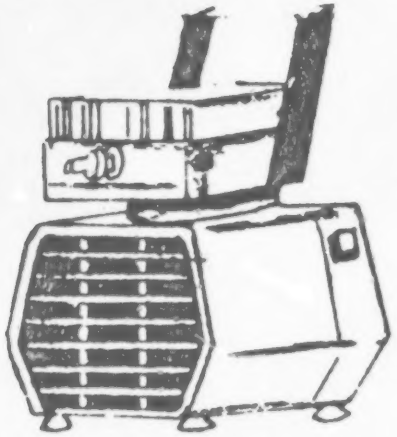


RECORDING MPN DATA

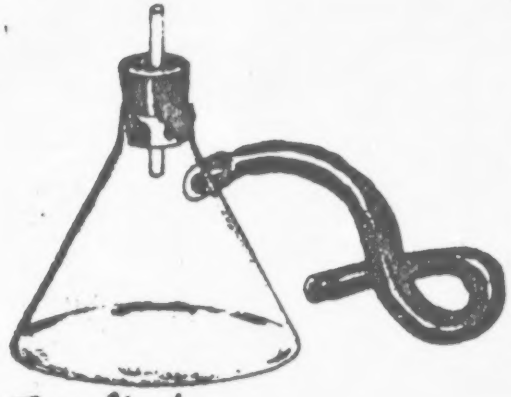
SAMPLE NUMBER	TUBE NUMBER	VOLUME INOCULATED	DATE INOCULATED	PRESUMPTIVE RESULTS		CONFIRMATORY RESULTS 48 HR.	TECHNICIANS INITIALS
				24 HR.	48 HR.		

MPN INDEX AND 95% CONFIDENCE LIMITS FOR VARIOUS COMBINATIONS OF POSITIVE AND NEGATIVE RESULTS WHEN FIVE 10-ML PORTIONS ARE USED

No. of Tubes Giving Positive Reaction out of 5 of 10 ml Each	MPN Index /100 ml	95% Confidence Limits	
		Lower	Upper
0	2.2	0	6.0
1	2.2	0.1	12.6
2	5.1	0.5	19.2
3	9.2	1.6	29.4
4	16.	3.3	52.9
5	16.	8.0	Infinite



Vacuum pump



Trap flask
Filtering flask
Vacuum tubing

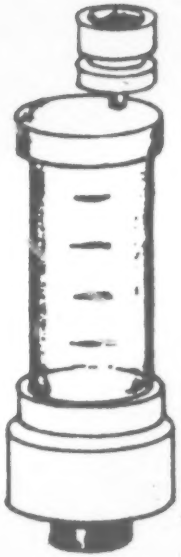
Glass or autoclavable plastic rinse bottle

Forcep

100 ml graduated cylinder

Membrane Filtration Equipment

Bunsen or Alcohol burner

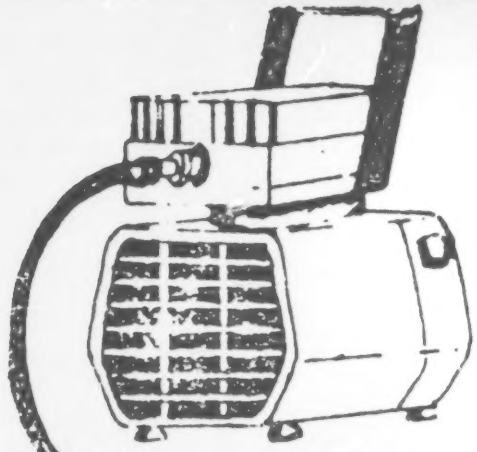


Filtering funnel. Borosilicate glass Rubber collar or clamp

Filtering funnel
Stainless steel
twist coupling

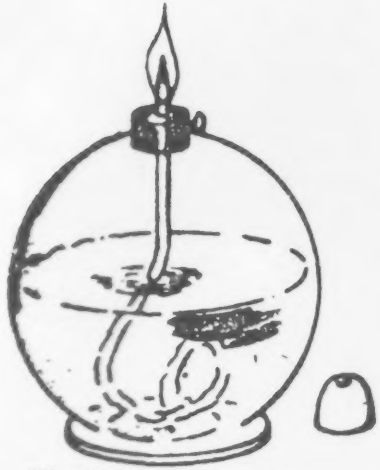
Filtering funnel
Polysulfone plastic
magnetic coupling

Membrane Filtration Equipment Assembly

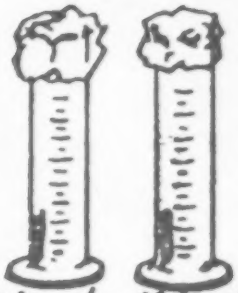


Vacuum pump

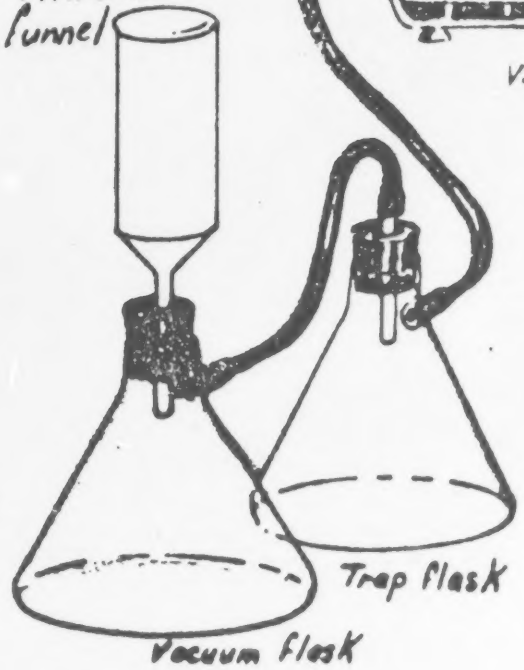
Filtration funnel



Akohol Burner



Sterile graduated 100 ml. cylinders

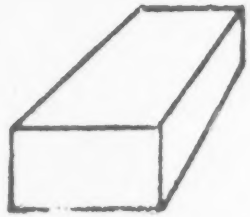


Trap flask

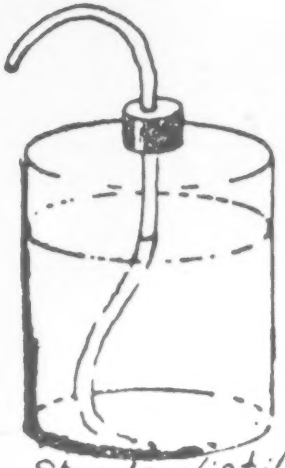
Vacuum flask

Forceps in 95% alcohol

Sterile 50mm x 12 mm Petri Dishes



Sterile membrane filters

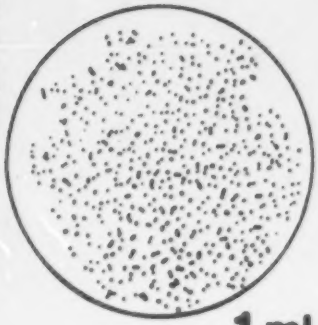


Sterile distilled buffered water in sterile rinse bottle.

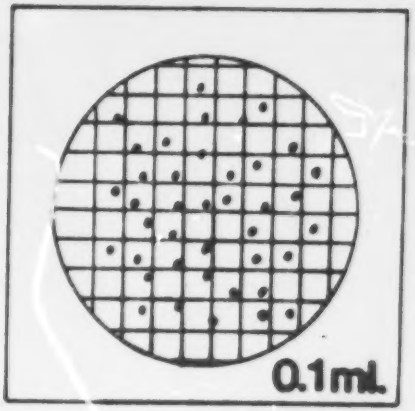
PLACEMENT OF MEMBRANE ONTO PLATE OF MEDIA

FROM:
SIMPLIFIED PROCEDURES FOR
WATER EXAMINATION - LAB. MANUAL
AWWA

CHOOSE THE CORRECT MEMBRANE



1 ml.

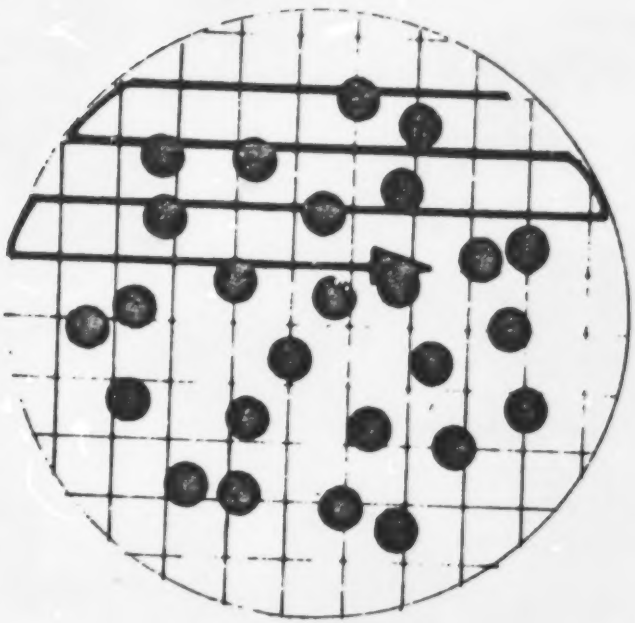


0.1ml.

0.01 ml.

CONTAINS 20 - 80 COLIFORM COLONIES, BUT FEWER THAN 200 COLONIES TOTAL.

Membrane Filter Counting Procedure



CALCULATIONS

Sample A		Sample B		Sample C	
Amt. Filtered	Count	Amt. Filtered	Count	Amt. Filtered	Count
100 mls.	64	100 mls.	107	100 mls.	4
50 mls.	26	50 mls.	53	10 mls.	0
10 mls.	4	10 mls.	14	1 mls.	0

$$\frac{\text{Count + count}}{\text{Total amt. filtered}} \times 100$$

Example:

$$\frac{64 + 26}{100 + 50} = \frac{90}{150} \times 100 = 60$$

Report as: 60/100 ml.

$$\frac{\text{Count}}{\text{Amt. filtered}} \times 100$$

Example:

$$\frac{53}{50} \times 100 = 106$$

Report as: 110/100 ml.

Report as: 4/100 ml.

Module No:	Module Title: Total Coliform Determination in Drinking Water
Approx. Time:	Submodule Title: Multiple Tube Technique
	EVALUATION - PART A

Objectives:

Upon completion of this module, the participant should be able to demonstrate the ability to perform a total coliform determination by the multiple tube technique and/or accurately answer 80% of the evaluation questions over the procedure.

EXAM QUESTIONS

Topic: Introduction

1. What does the presence of coliforms in a drinking water supply indicate with respect to chlorination?

2. If coliform bacteria are present in drinking water what other type of organisms of concern may also be present?

3. Describe the coliform group with respect to the following characteristics:

   a. _____ shaped

   b. gram _____

   c. Produce _____ when ferments lactose.

4. Using the Multiple Tube Technique, in order to meet the current water quality standards, not more than _____% of the portions tested in any month may show the presence of coliform bacteria.

Topic: Laboratory Equipment

1. What would an autoclave be used for?

2. Why use an incubator for growing bacteria?

3. State 2 reasons for using only a cotton plugged, sterile pipet when pipeting by mouth in a microbiology lab.

4. Why is it important to properly rinse glassware after washing?

5. What device is used to test the pH of growth media?

6. What device is used to transfer the bacteria from the positive presumptive test into the confirming media?

Topic: Laboratory and Media Preparation

1. State the 2 things a disinfectant is used for.
2. Why is the sample bottle wrapped before being sterilized?
3. State 2 ways to obtain distilled water.
4. What are the 2 chemicals that can be used to buffer sterile dilution water?
5. Is the growth media for this procedure sterilized in a hot air oven or autoclave?
6. What would an autoclave cycle of 15 min. at 121^{0} C (15 psi) with a rapid exhaust and a 10 min. allowance for drying be used for?
7. Where is sterile distilled buffered water stored.
8. Why must paper wrapped equipment remain dry after sterilization?
9. List the steps in proper glassware washing.
10. Is tap water of sufficient quality to use for growth media preparation?

Topic: Sampling

1. On what is the frequency of sampling based?
2. What chemical is used to dechlorinate a sample?
3. Why is a sampling tap flamed with a propane torch?
4. How long may a sample be held before it is tested for total coliform bacteria?
5. What happens to the bacterial population if the sample is not kept chilled?

Topic: Sample Dilution

1. Should sample dilution ever be necessary when sampling drinking water supplies?
2. Diagram how to get a 1:10000 dilution.

Topic: Multiple Tube Test Procedure and Data Interpretation

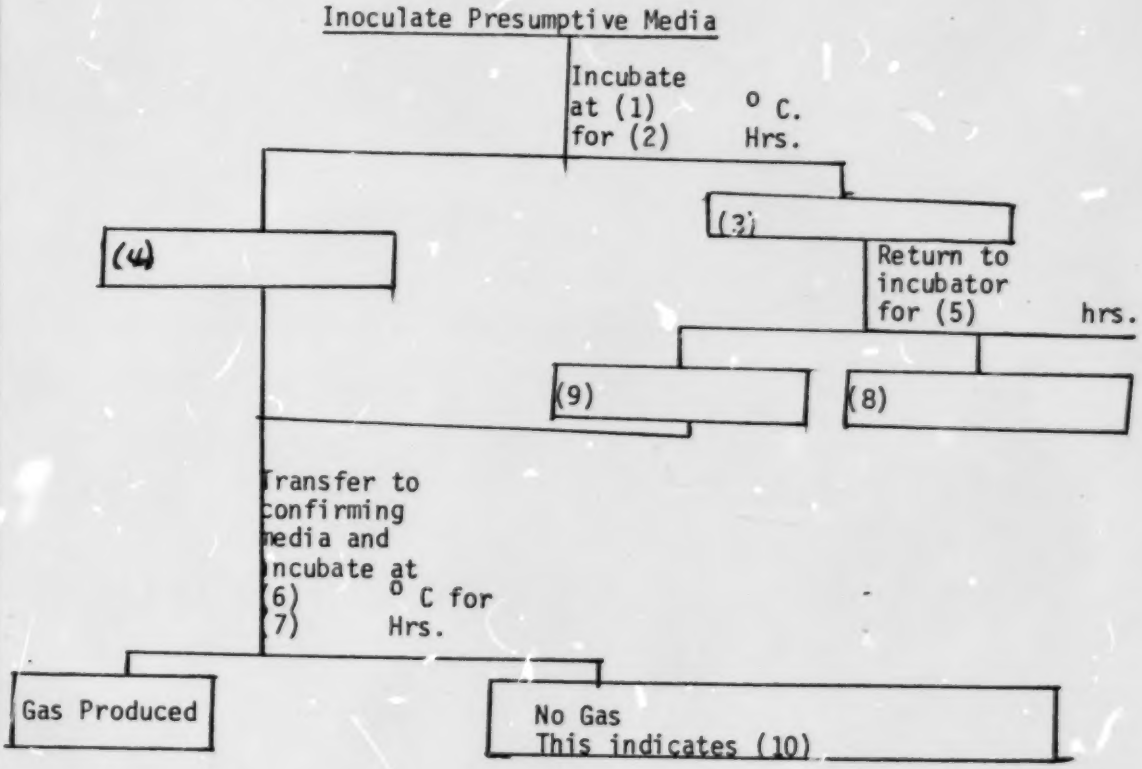
1. Why is the work area disinfected immediately before testing begins?
2. Which broth is inoculated directly with the water sample Lauryl Tryptose Sulfate or brilliant green bile?

3. At what temperature are the inoculated tubes incubated?

4. Is it necessary to use aseptic technique in planting the sample in the presumptive growth media transferring growth to the confirming media?

5. How are the old cultures processed before test tubes are washed?

6. Fill in the ten blanks with the correct information.



7. Given the following data sheet, does the water tested meet the standards for drinking water?

Sample Information

Test Results

Tube #	Presumptive	Confirmatory
1.	+	-
2.	+	-
3.	+	-
4.	- Not transferred	
5.	- Not transferred	

Module No:	EVALUATION - PART A

Instructor Notes:	Instructor Outline:
Answers Topic: Introduction 1. Chlorination has been insufficient. 2. Disease causing microorganisms 3. a. Rod b. Negative c. Gas 4. 10% Topic: Laboratory Equipment 1. Sterilizing heat stable equipment and liquids - or - Processing old cultures before disposal 2. It provides a controlled environment for the bacteria to grow in. 3. To protect the sample from contamination. To protect the lab technician from contamination. 4. Rinsing removes detergent residue which can inhibit bacterial growth. 5. a pH meter 6. a 3 mm. inoculating loop	Give the participants a sample to analyze by the multiple tube method and/or the total coliform - multiple tube evaluation questions to answer.

Module No:	EVALUATION - PART A

Instructor Notes:	Instructor Outline:

Topic: Laboratory & Media Preparation

1. General Laboratory Clean-up Cleaning up skilled bacterial cultures.

2. It allows the sample bottle to be stored without becoming contaminated.

3. Purchase a distillation unit and make it or purchase the distilled water from a reliable source.

4. Peptone
   $KH_2 PO_4$ (Potassium dihydrogen phosphate)

5. Autoclave

6. Sterilizing dry goods (i.e. glassware)

7. In the refrigerator

8. Bacteria is able to move through wet paper to contaminate the contents but not through dry paper.

9. (1) Wash in hot soapy water.
   (2) Rinse in hot tap water 6 - 12 times.
   (3) Rinse 1 - 3 times in distilled water
   (4) Air dry
   (5) If spots appear when dry, rewash.

10. No

Module No:	EVALUATION - PART A

Instructor Notes:	Instructor Outline:
Topic: Sampling 1. The population served 2. Sodium thiosulfate 3. To incinerate the bacteria on it. 4. 6 hours 5. It will change, first with growth followed by rapid die off.	
Topic: Sample Dilution 1. No 2. Sample — 1 ml. →	99 ml. dilution blank ↓ 1 ml. 99 ml. dilution blank This is the 1:10,000 dilution
Topic: Multiple Tube Test Procedure & Data Interpretation 1. Disinfection removes most dust and bacteria from the work area and this lowers the risk of contamination. 2. Lauryl Tryptose Sulfate Broth 3. 35 ± 0.5 ^{o} C 4. Yes 5. They are sterilized in an autoclave	

Module No:	EVALUATION - PART A

Instructor Notes:	Instructor Outline:
6. (1) 35 ± 0.5° C. (2) 24 hrs. (3) No gas (4) Gas produced (5) 24 hrs. (6) 35 ± 0.5° C. (7) 48 hrs. (8) No gas (9) Gas produced (10) No coliforms present	

Module No:	Module Title:
	Total Coliform Determination in Drinking Water
	Submodule Title:
Approx. Time:	Membrane Filter Technique
1 hour	EVALUATION - PART B

Objectives:

Upon completion of this module, the participant should be able to demonstrate the ability to perform a total coliform determination by the membrane filter technique and/or accurately answer 80% of the evaluation questions over the procedure.

EXAM QUESTIONS

Topic: Introduction

1. What does the presence of coliforms in a drinking water supply indicate with respect to chlorination?

2. If coliform bacteria are present in drinking water what other type of microorganisms of concern may also be present?

3. Describe the coliform group with respect to the following characteristics:

   a. Shape?

   b. Gram_____

   c. Produces _____when ferments lactose

   d. Develops a _____surface sheen when grown on m-Endo growth media

4. Using the membrane filter technique, the total coliform monthly average must not exceed_____ per_____mls. in order for the water to meet current water quality standards for drinking water.

Topic: Laboratory Equipment

1. What would an autoclave be used for?

2. Why use an incubator for growing bacteria?

3. List the 5 pieces of equipment in use when filtering a water sample.

4. State 2 reasons for using only a cotton plugged, sterile pipet when pipetting by mouth in a microbiological lab.

5. Why is it important to properly rinse glassware after washing?

6. Why must bacterial cultures be sterilized before being disposed of?

Topic: Laboratory Preparation

1. State the 2 things a disinfectant is used for.
2. Why is equipment packaged or wrapped before being sterilized?
3. State 2 ways to obtain distilled water.
4. What are the 2 chemicals that can be used to buffer sterile dilution water?
5. Is m-Endo growth media sterilized and why?
6. What would an autoclave cycle of 15 min. at 121° C. (15 psi) with a rapid exhaust and a 10 min. allowance for drying be used for?
7. Where is sterile distilled buffered water stored.
8. Why must paper wrapped equipment remain dry after sterilization?
9. List the steps in proper glassware washing.

Topic: Sampling

1. On what is the frequency of sampling based?
2. What chemical is used to dechlorinate a sample?
3. Why is a sampling tap flamed with a propane torch?
4. How long may a sample be held before it is tested for total coliform bacteria?
5. What happens to the bacterial population if the sample is not kept chilled?

Topic: Sample Dilution

1. Should sample dilution ever be necessary when sampling drinking water supplies?
2. Diagram how to get a 1:10000 dilution.

Topic: Membrane Filtration Procedure

1. Why is the work area disinfected immediately before testing begins?

2. Why is the filtering funnel rinsed with sterile distilled buffered water after the sample is filtered?

3. What traps the bacteria and provides a surface for colony growth when a sample is filtered.

4. How are sterile membrane filters handled.

5. Why is the culture dish inverted during incubation?

6. What is the proper incubation time and temperature for the total coliform test.

Topic: Counting Procedure & Data Interpretation and Evaluation

1. What is the proper counting range?

2. How often will a 100 ml. sample of drinking water give a count within the proper range.

3. Describe the appearance of a coliform colony on m-Endo growth media.

4. Give the formula for computing the number of coliform bacteria per 100 mls. sample.

Module No:	EVALUATION - PART B

Instructor Notes:	Instructor Outline:
Answers Topic: Introduction 1. Chlorination has been insufficient. 2. Disease causing microorganisms 3. a. Rod shaped b. Negative c. Gas d. Golden-green metallic 4. 1 per 100 mls. Topic: Laboratory Equipment 1. Sterilizing heat stable equipment and liquids. - or 2. It provides a controlled environment for the bacteria to grow in. 3. Vacuum source Vacuum tubing Filtering flask Filtering funnel Membrane filter 4. To protect the sample from contamination 5. Rinsing removes detergent residue which can inhibit bacterial growth. 6. Sterilizing kills the bacteria thereby protecting the environment when the cultures are disposed of.	Give the participants a sample to analyze by the membrane filter method and/or the total coliform, membrane filter, evaluation questions to answer. 1. Sterilizing spent cultures before disposing of them.

Module No:	EVALUATION - PART B

Instructor Notes:	Instructor Outline:

Answers

Topic: Laboratory Preparation

1. (1) General laboratory clean-up
   (2) Cleaning up spilled bacterial cultures

2. It allows the equipment to be stored without becoming contaminated.

3. (1) Purchase a distillation unit and make it.
   (2) Purchase the distilled water from a reliable source.

4. (1) Peptone
   (2) $KH_2 PO_4$ (Potassium dihydrogen phosphate)

5. m-Endo growth media is not sterilized because it contains heat sensitive components which will be destroyed at sterilizing temperatures.

6. Sterilizing dry goods, glassware, or other equipment.

7. In the refrigerator

8. Bacteria is able to move through wet paper to contaminate the contents but not through dry paper.

9. (1) Wash in hot soapy water
   (2) Rinse in hot tap water 6 - 12 times.
   (3) Rinse 1 - 3 times with distilled water
   (4) Air dry
   (5) If spots appear when dry, rewash

Module No:	EVALUATION - PART B

Instructor Notes:	Instructor Outline:

Answers

Topic: Sampling

1. The population served
2. Sodium Thiosulfate
3. To incinerate the bacteria on it.
4. 6 hours
5. It will change, first with growth followed by rapid die off.

Topic: Sample Dilution

1. No
2. Sample — (1 ml.) → 100 ml. dilution blank

↓ 1 ml.

This is the 1:10000 dilution → 100 ml. dilution blank

Topic: Membrane Filtration Procedure

1. Disinfection removes most dust and bacteria from the work area and this lowers the risk of contamination.
2. The rinses remove the bacteria which adhered to the sides of the funnel and deposits them on the membrane filter.
3. The membrane filter.
4. Membrane filters are handled by flamed forceps on the outer 1/8 inch.

Module No:	EVALUATION - PART B

Instructor Notes:	Instructor Outline:

Answers

5. To keep the moisture under the membrane. If it collects on the lid, it will drip onto the membrane surface and distort the colony growth.

6. 35 ± 0.5° C. for 22 - 24 hrs.

Topic: Counting Procedure & Data Interpretation and Evaluation

1. 20 - 80 coliform colonies with no more than 200 total colonies.

2. Almost never

3. Dark red with a golden green metallic sheen.

4. $\frac{\text{count}}{\text{amount filtered}} \times 100$